Leibniz-Gemeinschaft

Leibniz-Institut für Polymerforschung Dresden e. V.

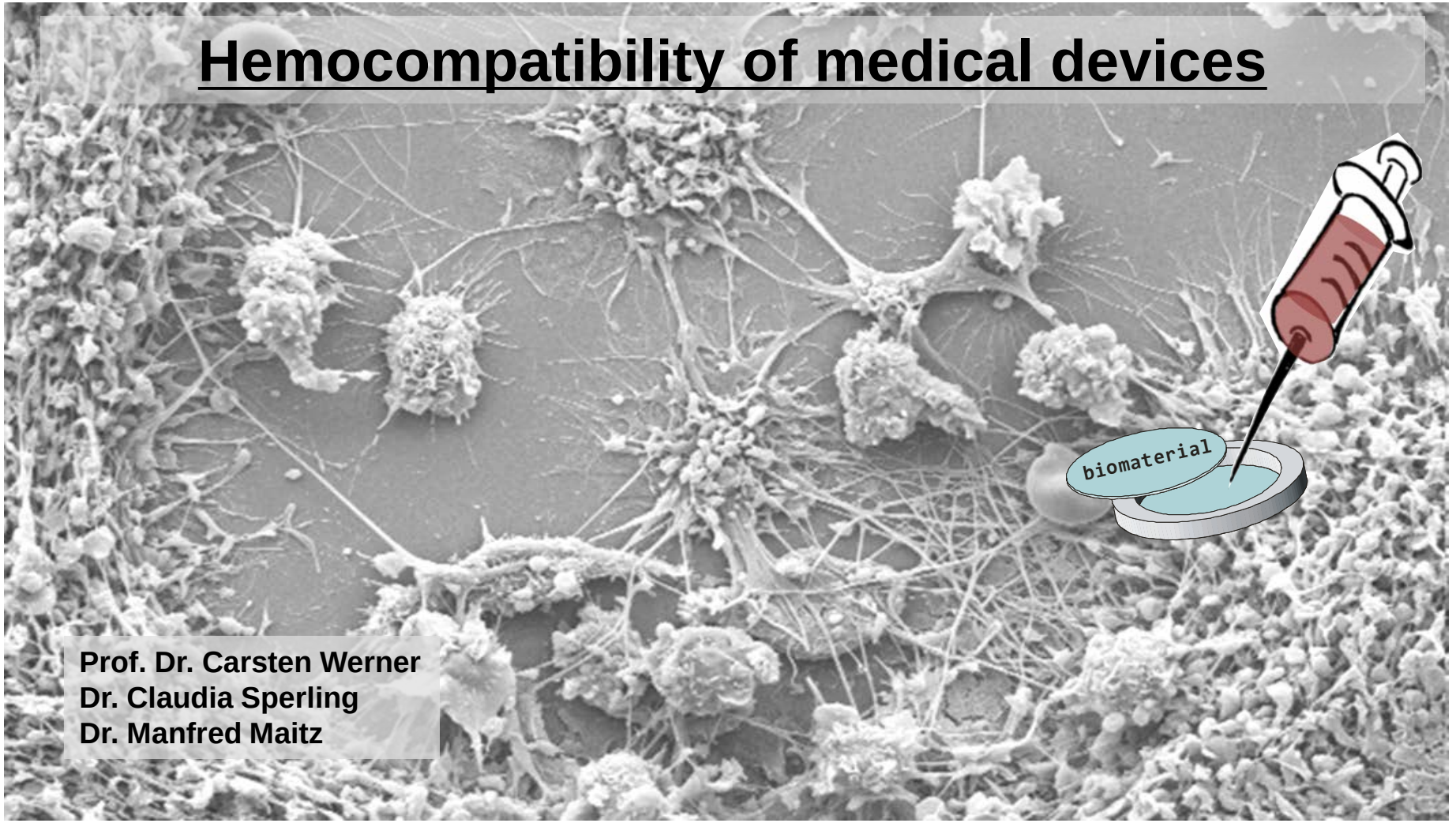
DRESDEN concept

CRTD Center for Regenerative Therapies TU Dresden

TECHNISCHE UNIVERSITÄT DRESDEN

MAX BERGMANN center of biomaterials dresden

Hemocompatibility of medical devices

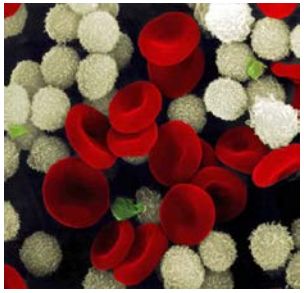


Prof. Dr. Carsten Werner
Dr. Claudia Sperling
Dr. Manfred Maitz

outline

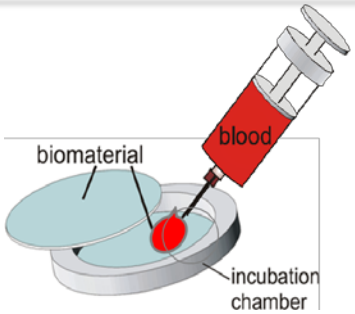
basics

- blood
- blood reactions on biomaterial surfaces
- hemocompatibility



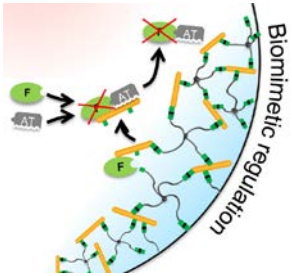
our research

- in vitro blood incubation
- initiation of blood activation on biomaterial surfaces



functionalization strategies

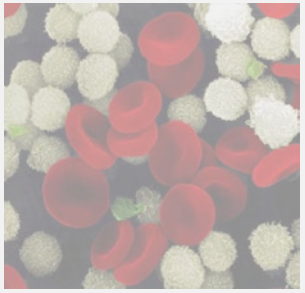
- passivation
- active interface



part 2

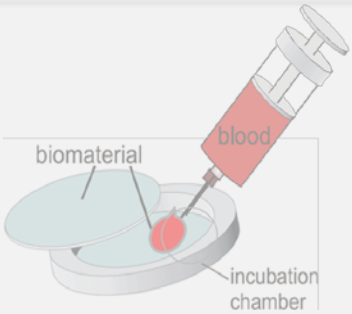
basics

- blood
- blood reactions on biomaterial surfaces
- hemocompatibility



our research

- in vitro blood incubation
- initiation of blood activation on biomaterial surfaces



functionalization strategies

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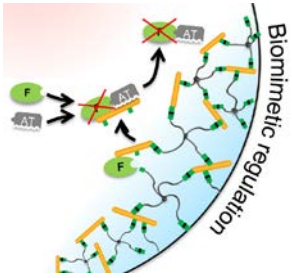
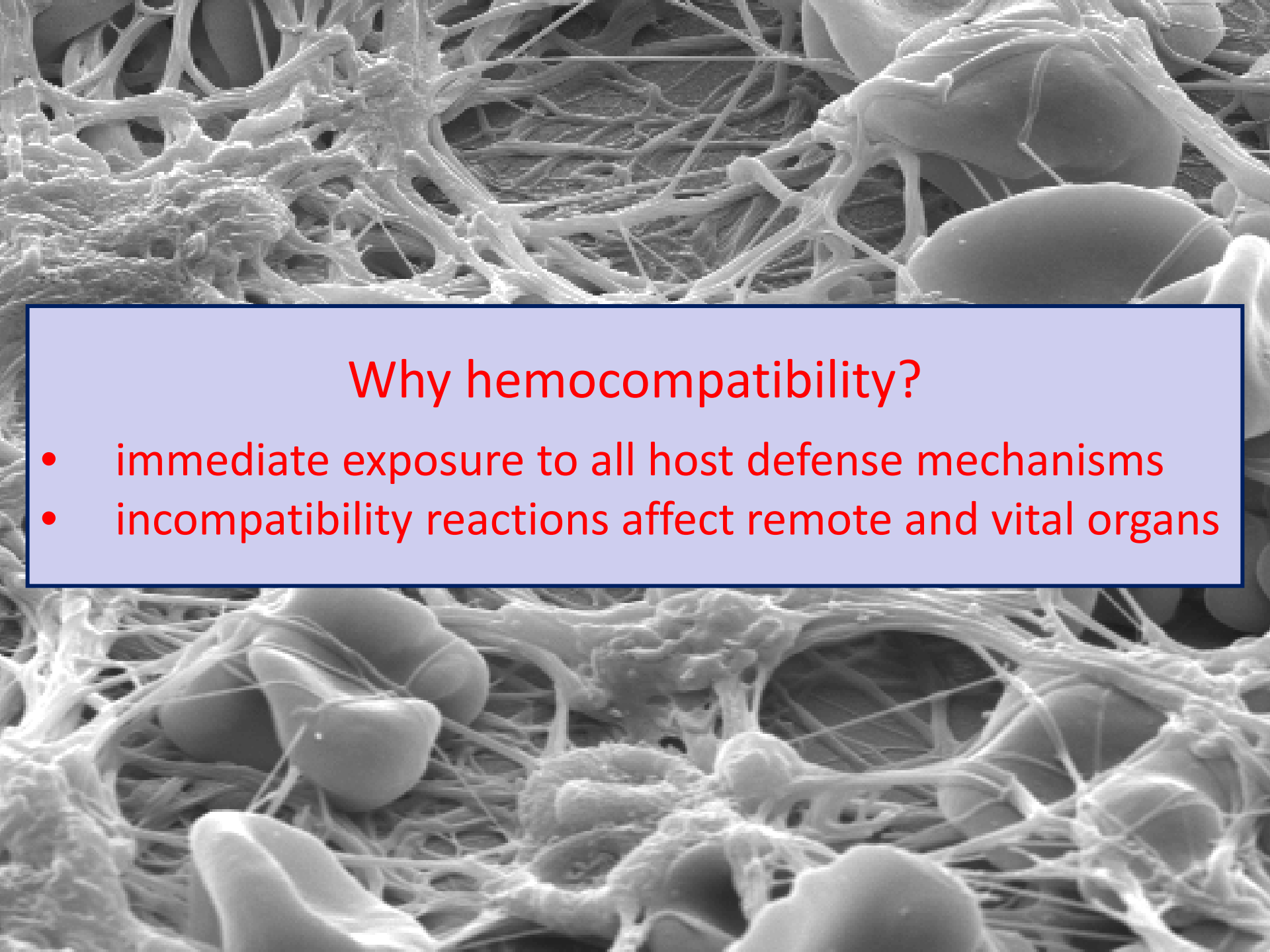


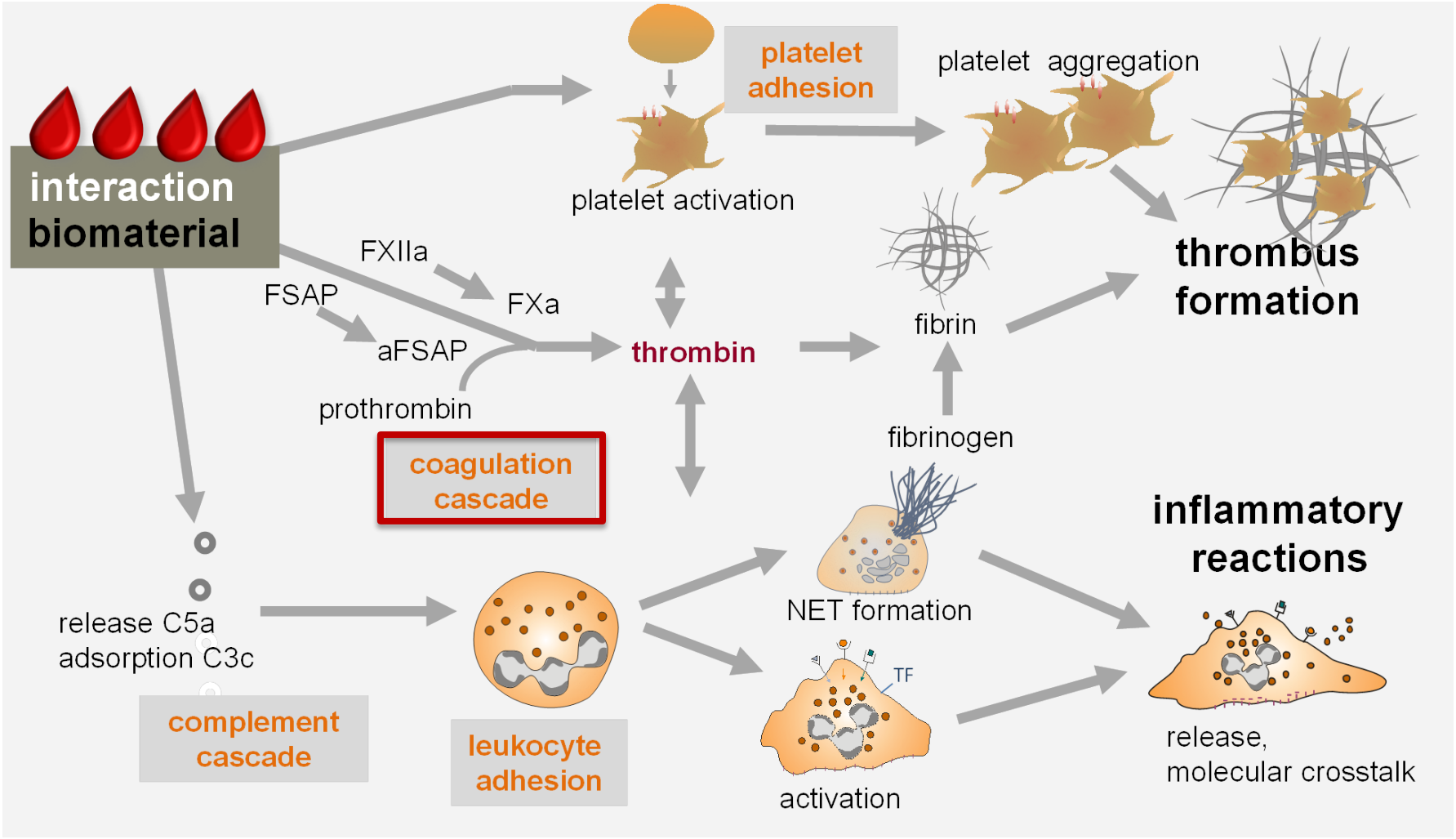


Why hemocompatibility?

- immediate exposure to all host defense mechanisms
- incompatibility reactions affect remote and vital organs

Blood reaction cascades

Primary aim: minimizing blood coagulation



Surface functionalization for hemocompatibility

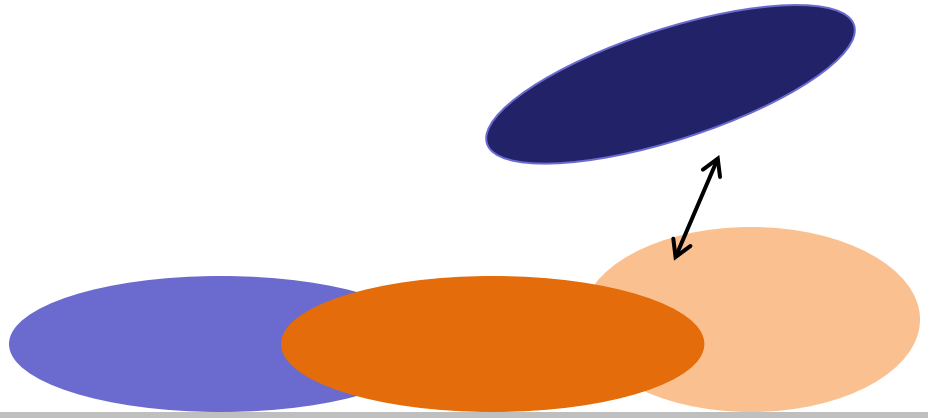
passivation - active inhibition
permanent - renewable

Surface functionalization for hemocompatibility

passivation - active inhibition
permanent - renewable

protein adsorption

‚translates‘ *the presence of a foreign surface into the ‚language‘ of the living organism*



repetition - protein adsorption

- albumin and fibrinogen show competitive behaviour
  - albumin/fibrinogen ratio frequently used for a first rating of hemocompatibility
  - *in vitro* dependent from the salt concentration and buffer system
- different behaviour of free and adsorbed fibrinogen
  - free fibrinogen without effect on blood platelets
  - adsorbed and denatured fibrinogen activates blood platelets
- threshold surface fibrinogen concentration for platelet activation at polymer surfaces
  - 30 ng/cm^2

hydrophilicity

hydrophobic hydrophilic

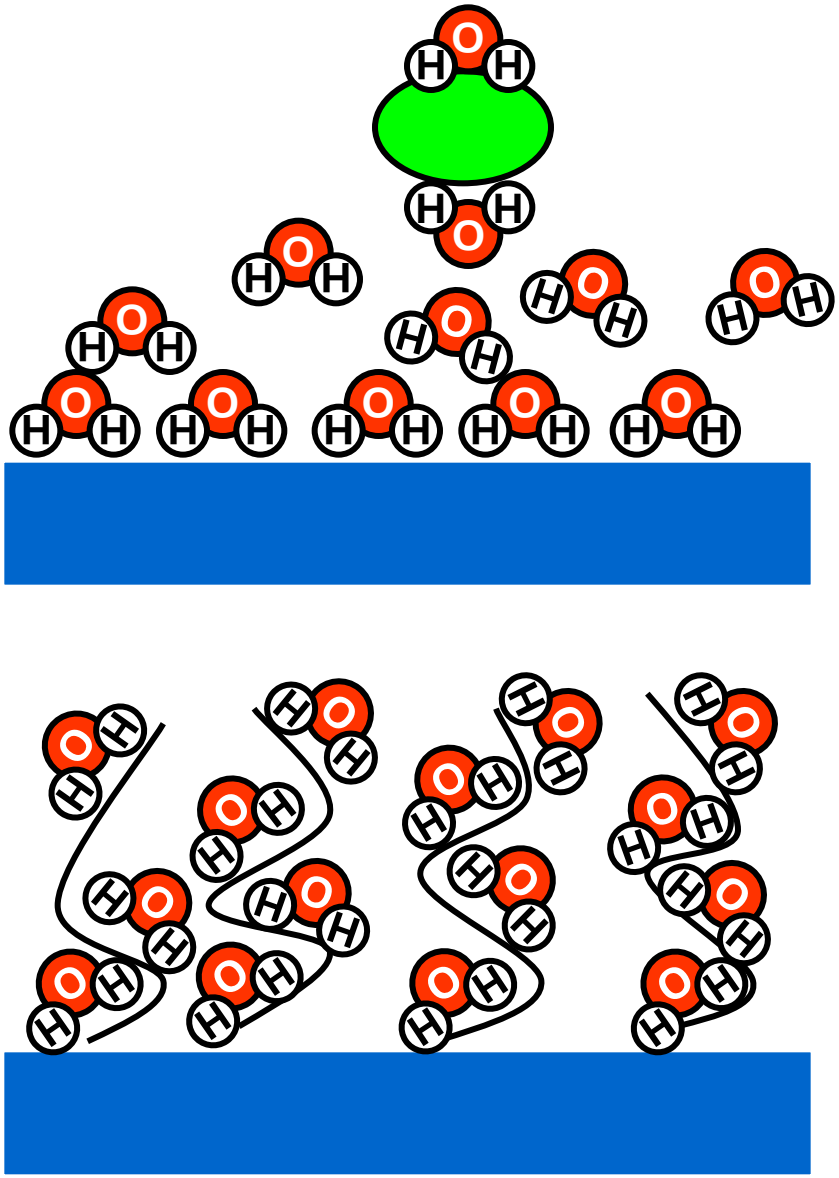
hydrophobic surfaces

- aliphatic groups $–CH_2\text{-}CH_3$
- aromatic groups
- inorganic carbon
- fluorine groups (Teflon) $–(C_2F_4)\text{-}$

hydrophilic surfaces

- ionized surfaces (acidic – alkaline)
- polar chemical groups
  - Ester R-COOR
  - Ether -C-O-C-
  - Alcohols C-OH
- zwitterionic materials
- pure metals (free electrons)

strategy 1: hydrophilicity



hydrophilic surfaces

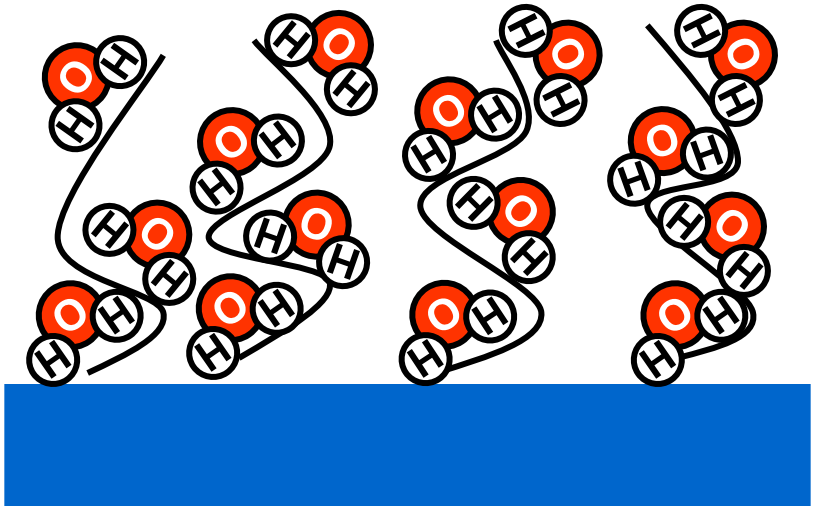
- adsorbed water makes them more or less invisible for biosystems

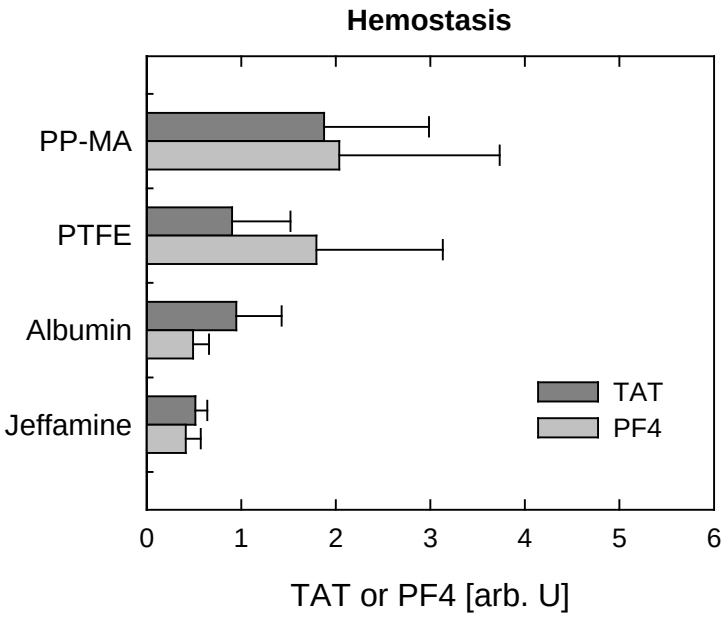
⇨ lower protein adsorption

⇨ less conformation changes of proteins

- effect is enhanced by flexible hydrophilic chains on the surface

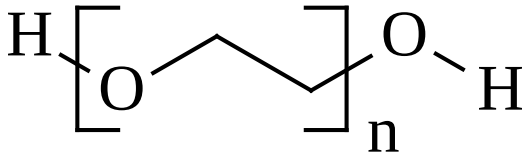
hydrophilicity



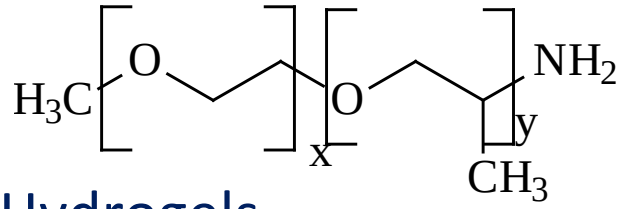


hydrophilic brushes

- Arrangement of water molecules at flexible hydrophilic chains
- Polyethylenglycol

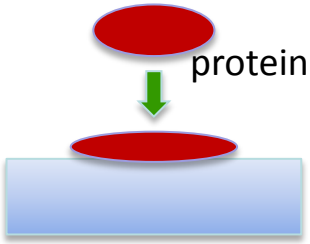


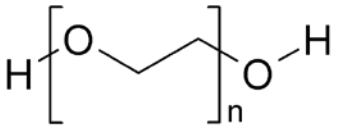
- Jeffamine®



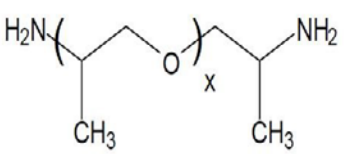
- Hydrogels

hydrophilic brushes prevent surface-protein interaction

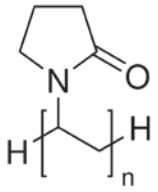




Poly(ethylen-glycole) PEG

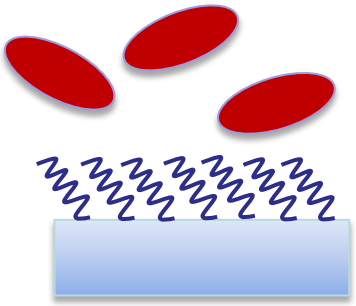


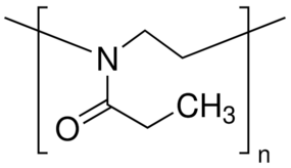
Jeffamine



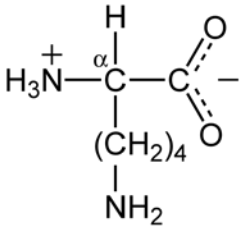
Poly(vinylpyrroli-done) PVP

protein

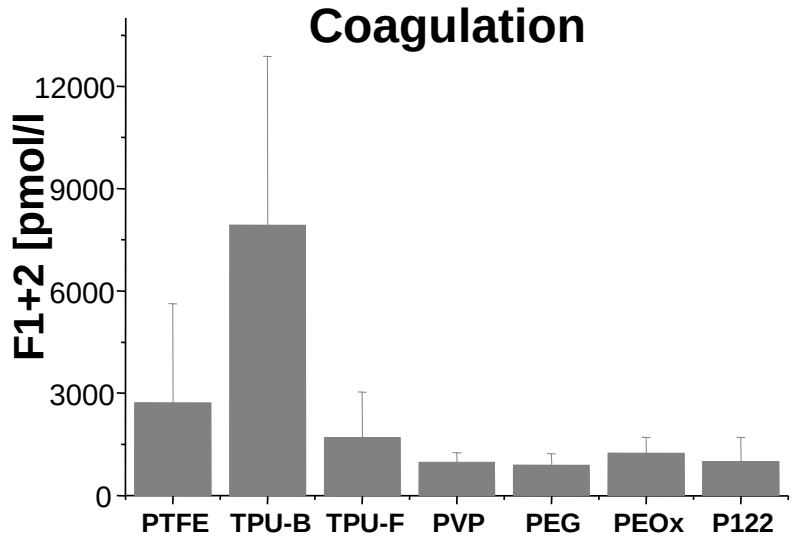


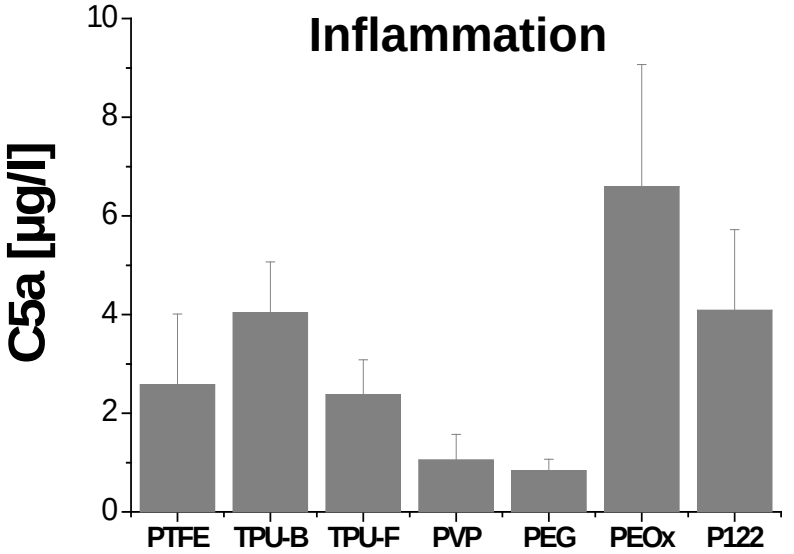


Poly(ethyl-oxazoline) PEOx



Zwitterion P122



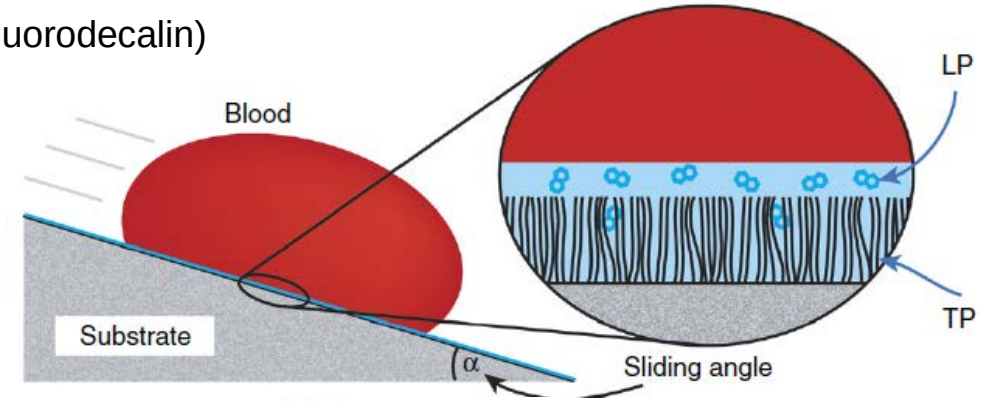


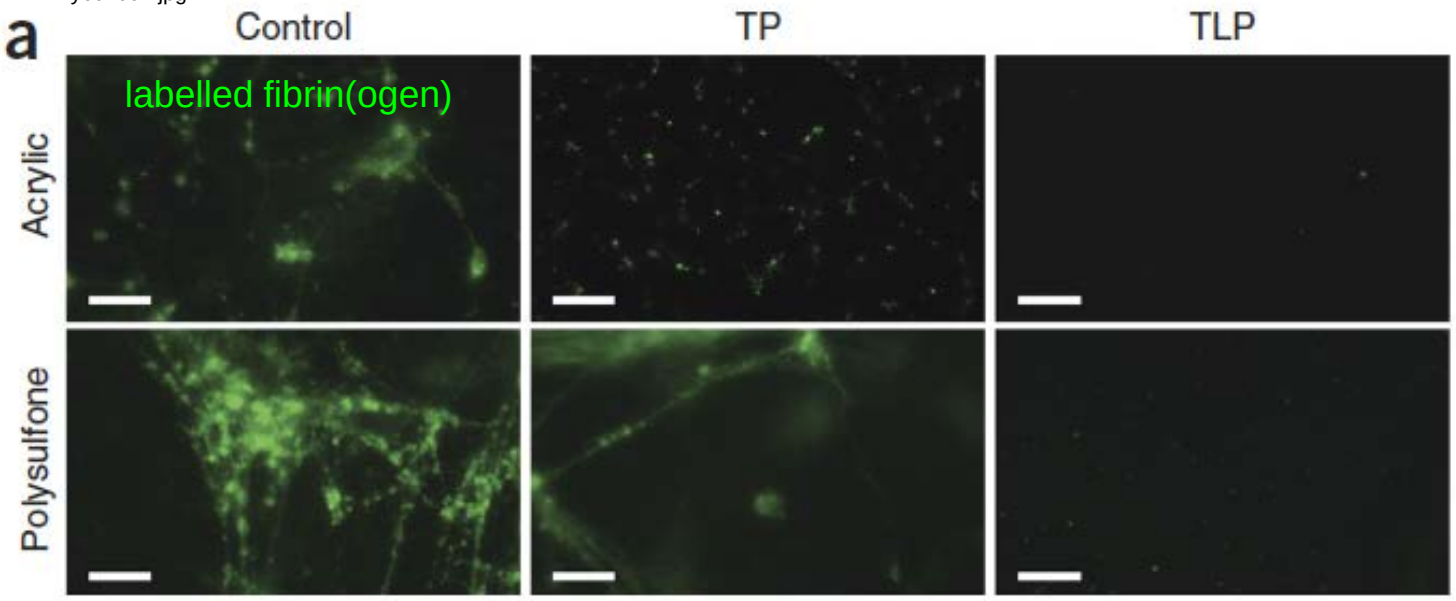
strategy 2: omniphobicity

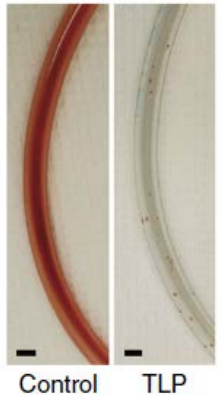
http://www.sarracenia.com/photos/nepenthes/nepenhyb02001.jpg

SLIPS: slippery, liquid-infused, porous surface

TP: tethered perfluorocarbon
LP: mobile layer of an LP (perfluorodecalin)



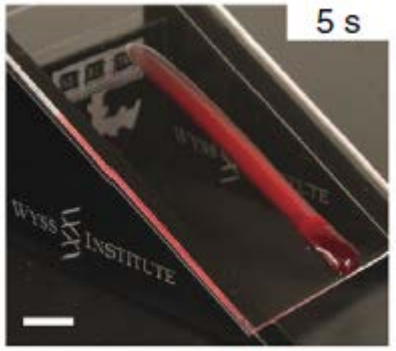




cardioperfusion tubing + porcine blood for 2 min.

D.C. Leslie, et al. **A bioinspired omniphobic surface coating on medical devices prevents thrombosis and biofouling**, Nature Biotechnology (2014).

strategy 2: omniphobicity



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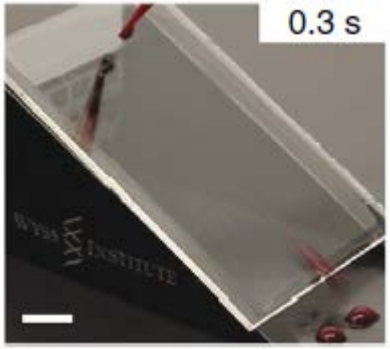
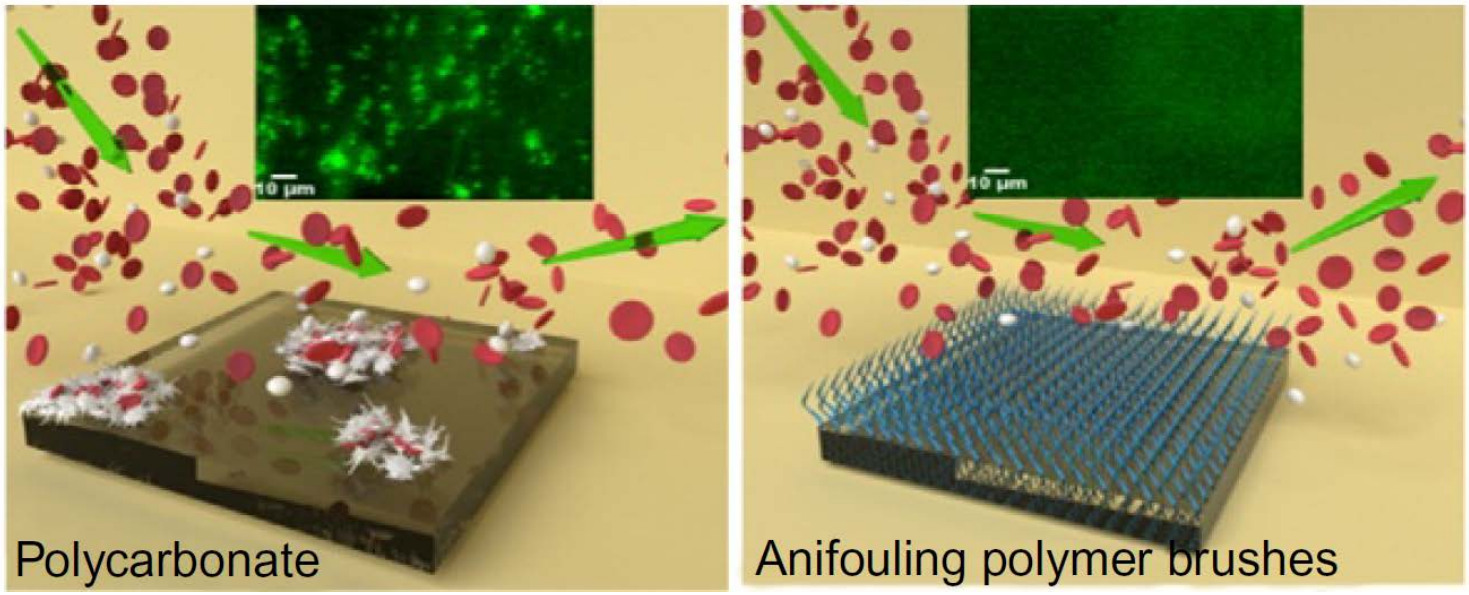


D.C. Leslie, et al. **A bioinspired omniphobic surface coating on medical devices prevents thrombosis and biofouling**, Nature Biotechnology (2014).

strategy 3: antifouling polymer brushes

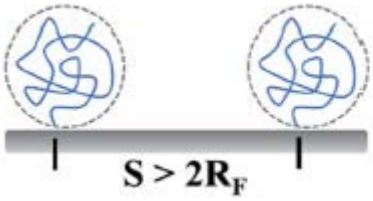


polymerbrushes on polycarbonate surfaces:
pronounced resistance to protein adsorption and marked reduction in thrombogenicity in relation to control substrate

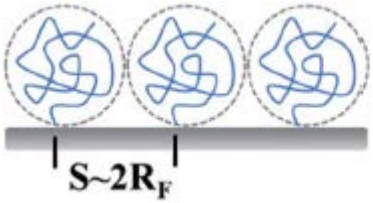
underlying principle: dependency on chain length and brush density determines mobility and resulting steric compulsion

A. de Los Santos Pereira, et al. Antifouling polymer brushes displaying antithrombogenic surface properties. Biomacromolecules 17, (2016)

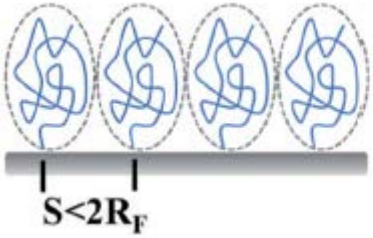
strategy 3: antifouling polymer brushes



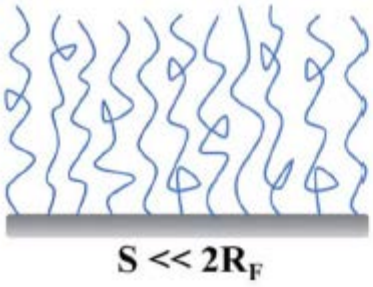
Non-overlapping mushrooms



Close packed mushrooms



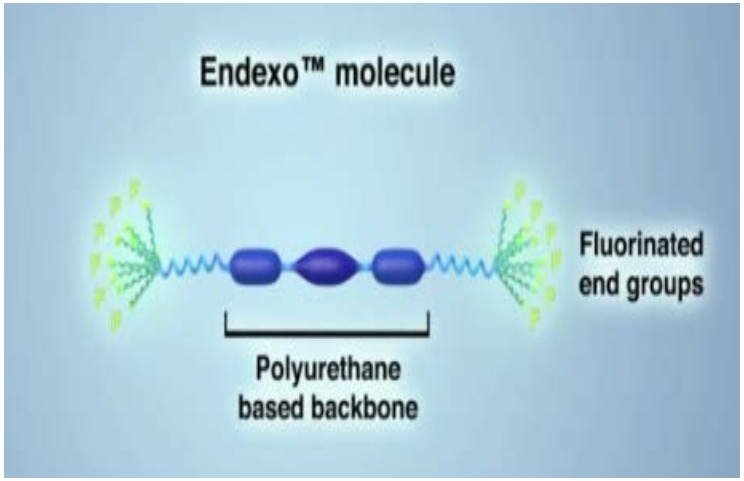
Extended mushrooms,
Dilute brush regime

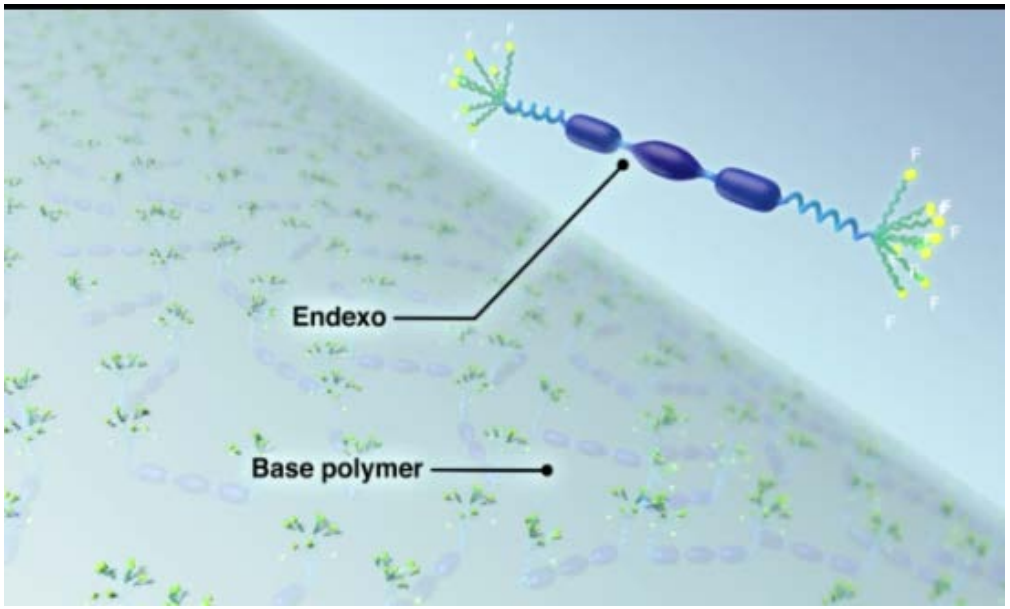


Highly extended chains,
Dense brush regime

X.L. Liu, L. Yuan, D. Li, Z.C. Tang, Y.W. Wang, G.J. Chen, H. Chen, J.L. Brash, Blood compatible materials: state of the art, Journal of Materials Chemistry B 2(35) (2014) 5718-5738.

strategy 4: surface modifying additives





J. Paul Santerre
Toronto, ON

www.interfacebiologics.com

M.L. Lopez-Donaire, J.P. Santerre, Surface modifying oligomers used to functionalize polymeric surfaces: Consideration of blood contact applications, Journal of Applied Polymer Science 131(14) (2014)

strategy 4: surface modifying additives

www.interfacebiologics.com/endexo.htm

RealPlayer-Konverter

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ibi INTERFACE biologics
A Material Advantage

COMMERCIAL APPLICATION CASE STUDY:

BIOFLO™ WITH ENDEXO FROM ANGIODYNAMICS

CLINICAL DATA

Average reduction across 8500 BioFlo PICCs studied:
As reported by AngioDynamics

50% ↓ DEEP VEIN THROMBOSIS

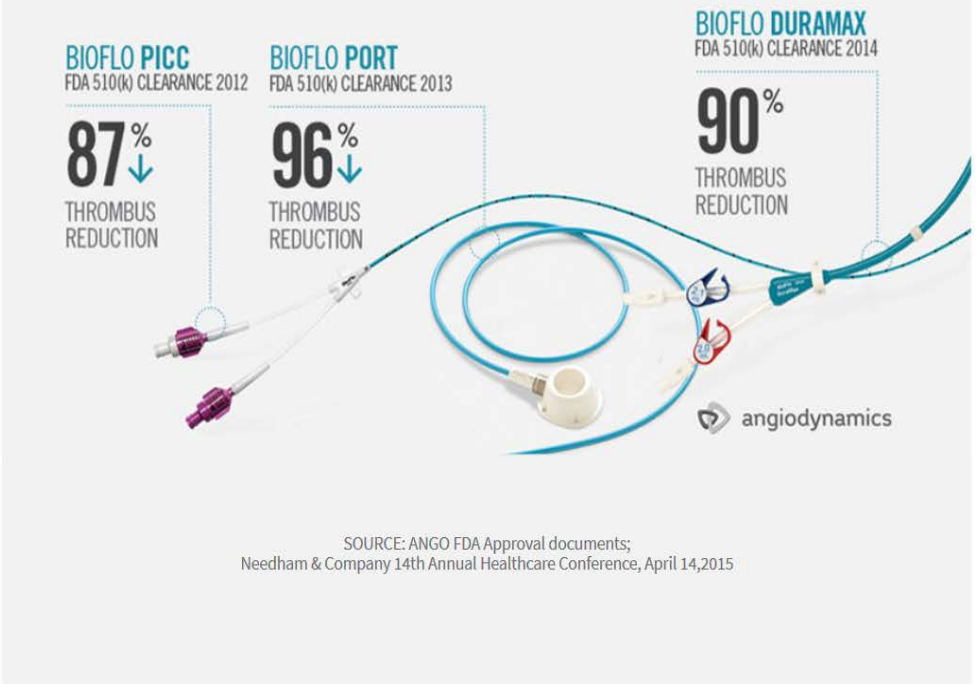
59% ↓ TISSUE PLASMINOGEN ACTIVATOR

COMMERCIAL TRACTION

+65% BIOFLO GROWTH

600+ BIOFLO CATHETERS

3700+ GPO HOSPITALS ELIGIBLE TO BUY



SOURCE: ANGO FDA Approval documents;
Needham & Company 14th Annual Healthcare Conference, April 14,2015

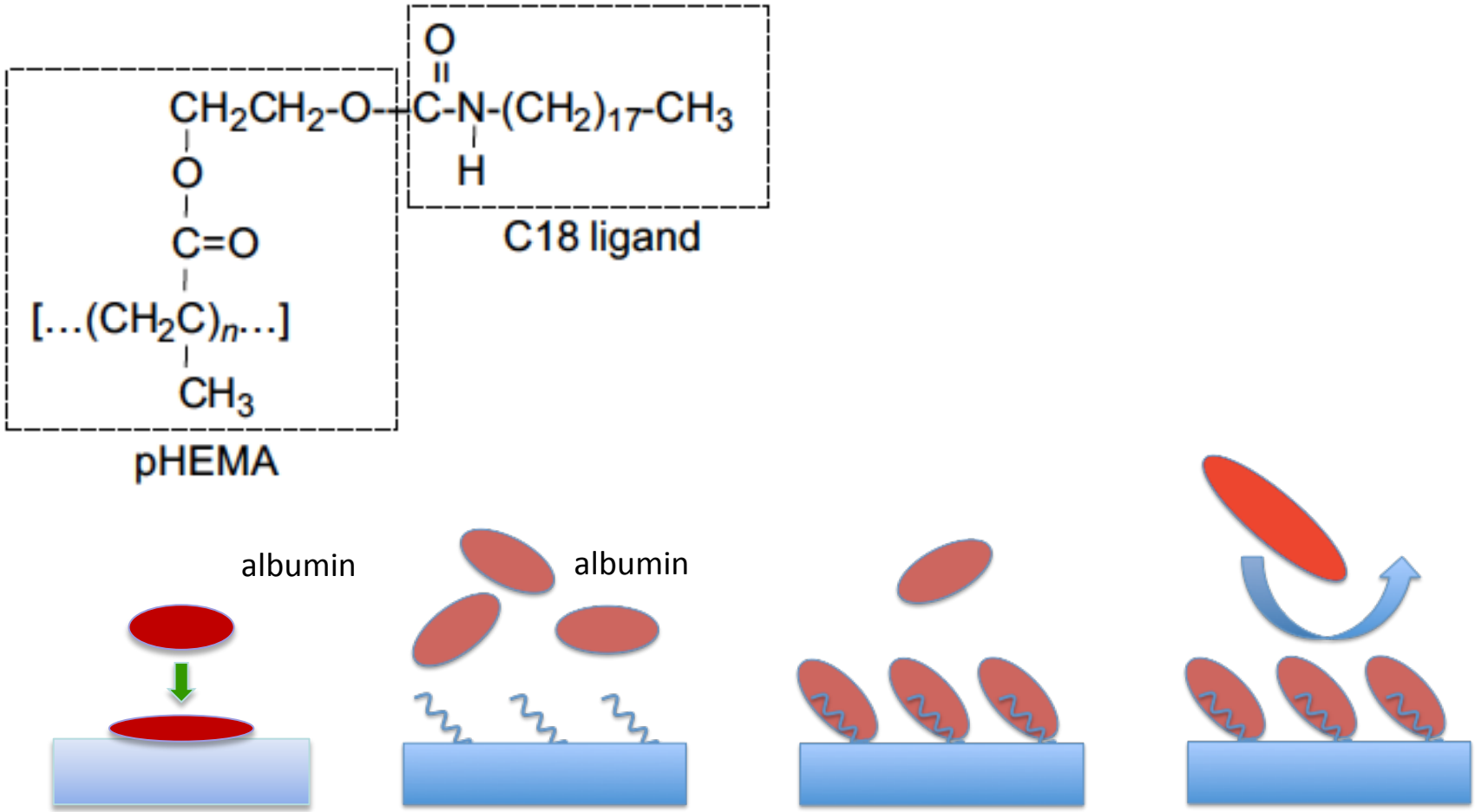
http://www.interfacebiologics.com/endexo.htm

Surface functionalization for hemocompatibility

passivation - active inhibition

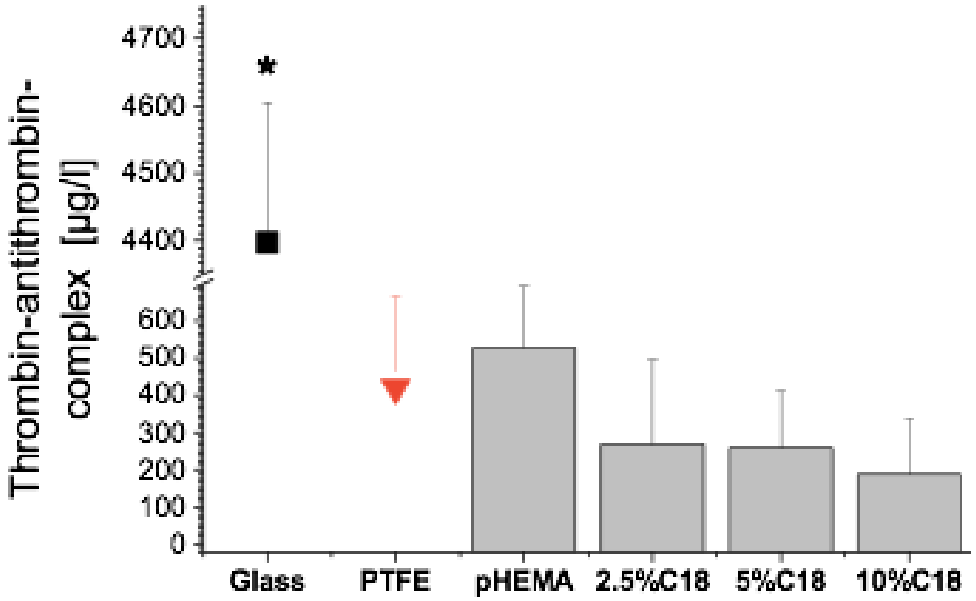
permanent - renewable

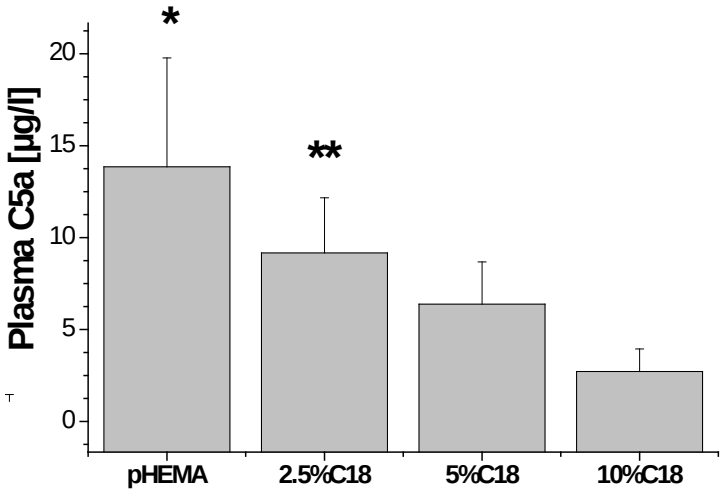
alkyl-grafted hydrogels for self-renewing albuminization

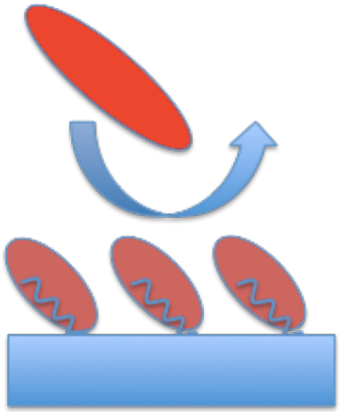


M. Fischer, C.P. Baptista, I.C. Goncalves, B.D. Ratner, **C. Sperling, C. Werner**, C.L. Martins, M.A. Barbosa, The effect of octadecyl chain immobilization on the hemocompatibility of poly (2-hydroxyethyl methacrylate), Biomaterials 33(31) (2012) 7677-85.

Alkyl-grafted hydrogels for self-renewing albuminization







challenges of passive modifications

- coagulation cascade, blood platelets and inflammation must be considered at the same time
- translation of effective surface characteristics into STABLE real world materials

Surface functionalization for hemocompatibility

passivation - active inhibition

permanent - renewable

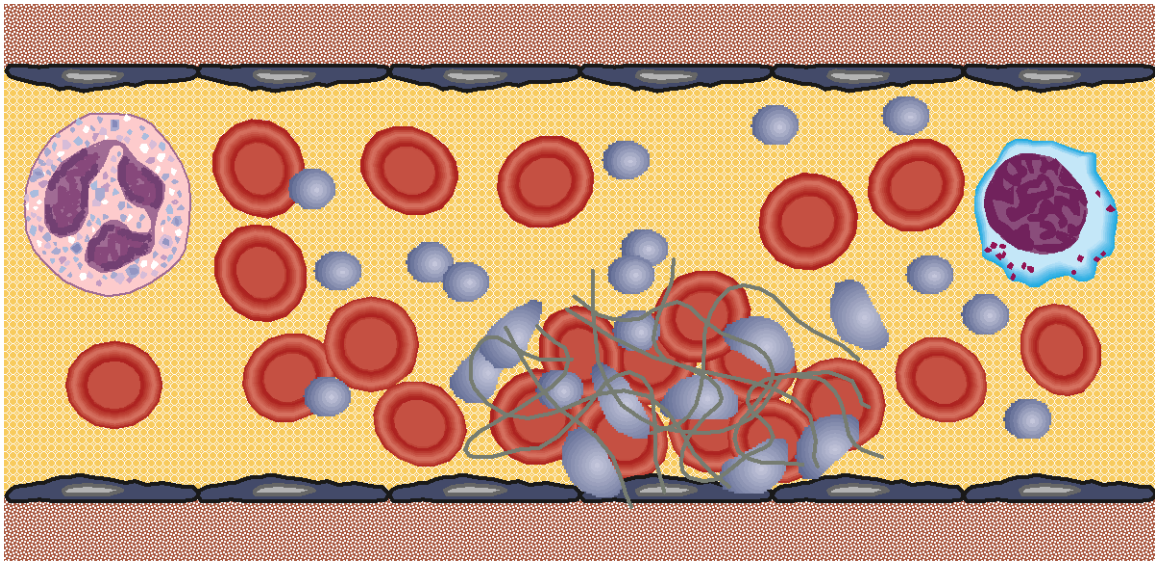
model surface: endothelium

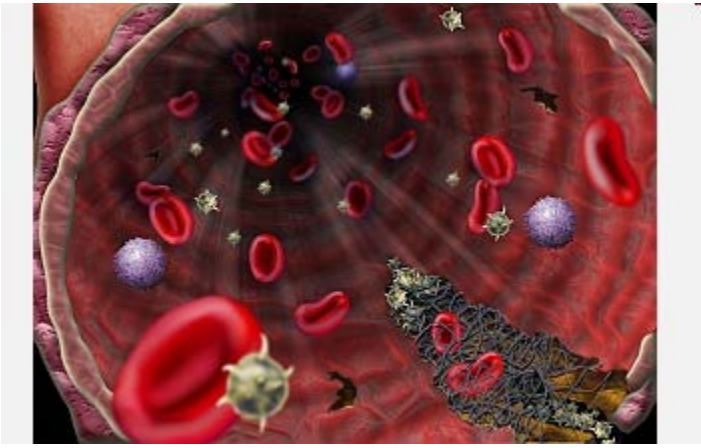
passive

- "appropriate" wettability
  - no protein adsorption
- smooth
- barrier against surrounding tissue

active (constant or regulated)

- anticoagulants
  - heparan sulfate
  - tissue factor pathway inhibitor (TFPI)
  - thrombomodulin
- anti platelet action
  - nitric oxide
- stimulated fibrinolysis
  - tissue plasminogen activator (tPA)





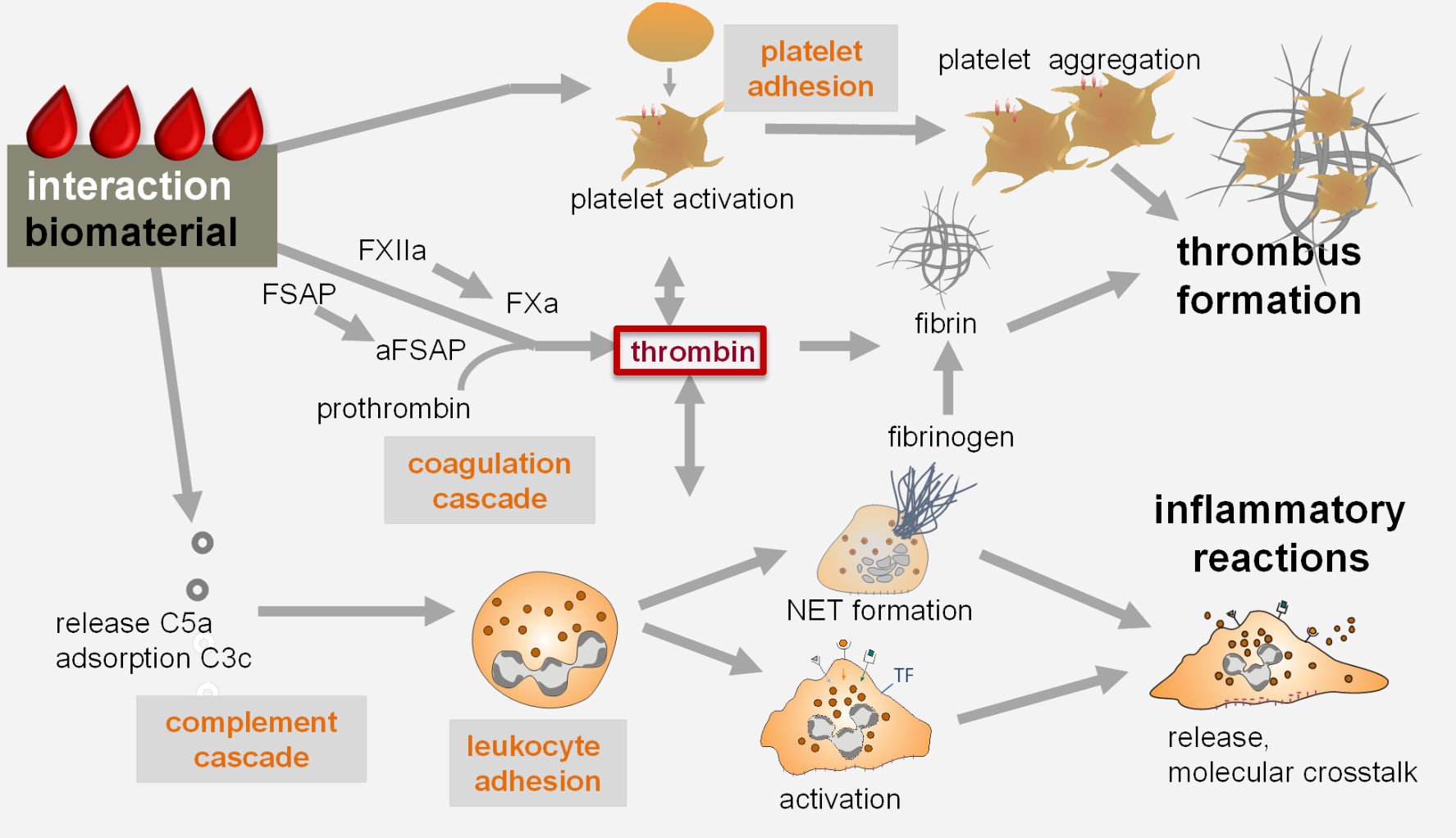
http://nsf.gov/

coatings to promote endothelialization

- direct seeding of endothelial cells (EC) (ex vivo)
- surface modification:
  - positive charge
  - immobilization of cell recognition sites
  - immobilization of ECM derived proteins / peptides
  - employment of growth factors (VEGF)
- in vivo endothelialization
  - EC migration to (modified) surfaces
  - capture of EPCs (endothelial progenitor cells)
    - via monoclonal antibodies
    - aptamers

No sufficiently reliable strategy with success in a clinical study found yet.

inhibition sites



thrombin inhibition

coatings based on natural substances

- **thrombomodulin** (complex formation with thrombin)
- **heparin** (activation of antithrombin AT)
- **hirudin** (direct thrombin inhibition)

coatings based on synthetic inhibitors

- **small molecule synthetic inhibitors**

Vessel wall protein thrombomodulin

Different functions within one molecule:

- Binds thrombin and changes its substrate specifity from fibrinogen to protein C

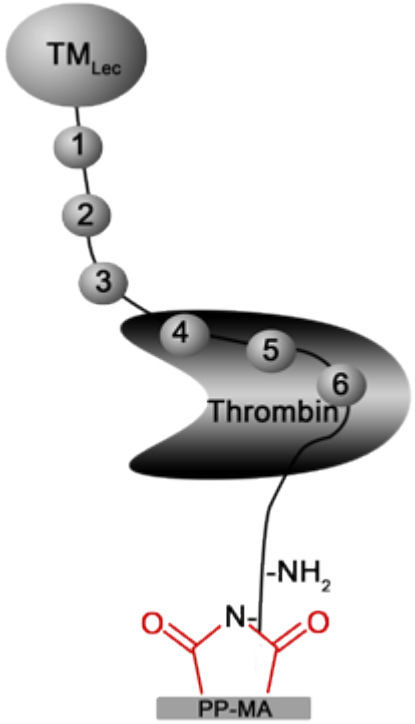
⇨ Inhibits coagulant thrombin activity

⇨ Activates anticoagulant protein C pathway

  – Inactivates Factor V and VIII

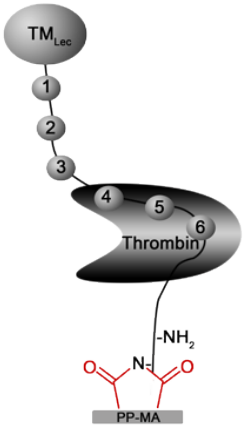
  – Antiinflammatory properties

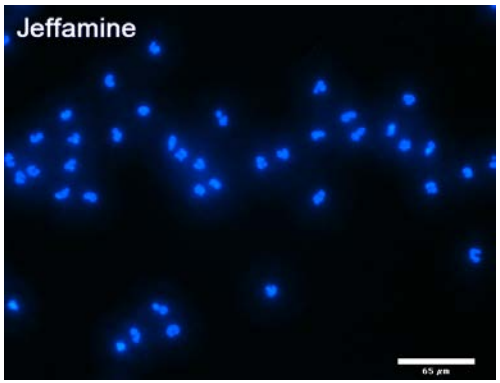
⇨ Leading anticoagulant and antiinflammatory molecule in the endothelium cell membrane

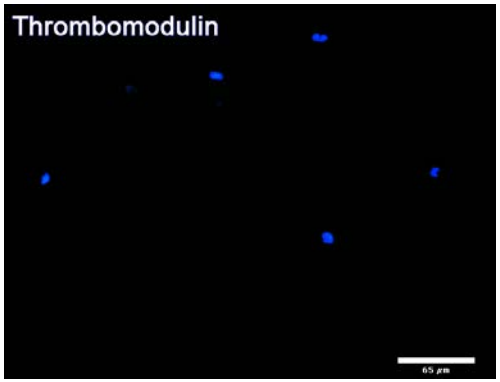


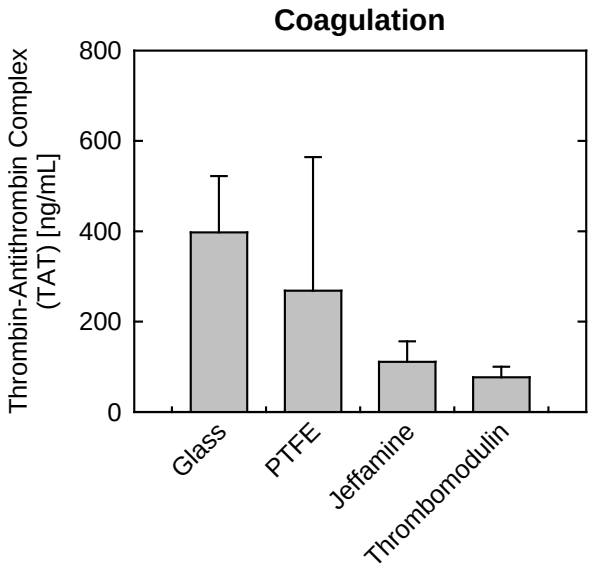
Vessel wall protein thrombomodulin

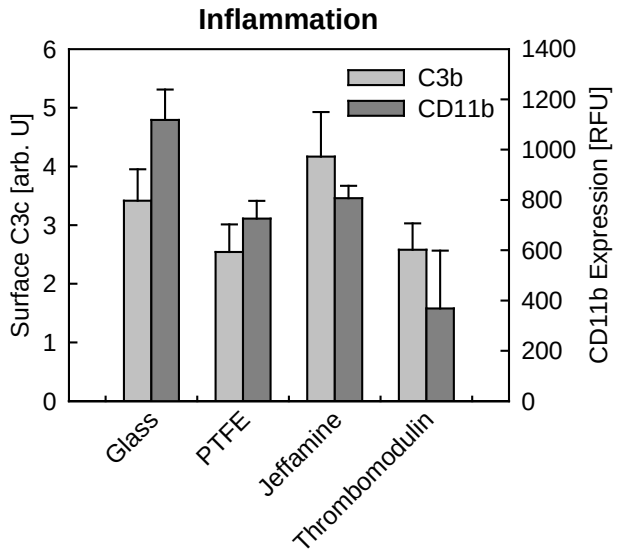
Immobilization in physiological orientation on model substrates











Reduced leukocyte adhesion with thrombomodulin

Less coagulation and inflammation than the controls PTFE or jeffamine.

thrombin inhibition

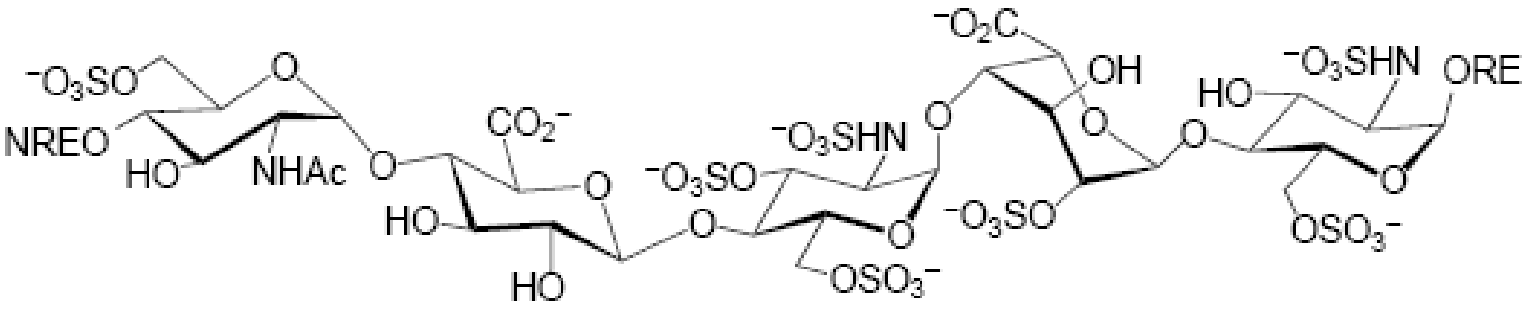
coatings based on natural substances

- **thrombomodulin** (complex formation with thrombin)
- **heparin** (activation of antithrombin AT)
- **hirudin** (direct thrombin inhibition)

coatings based on synthetic inhibitors

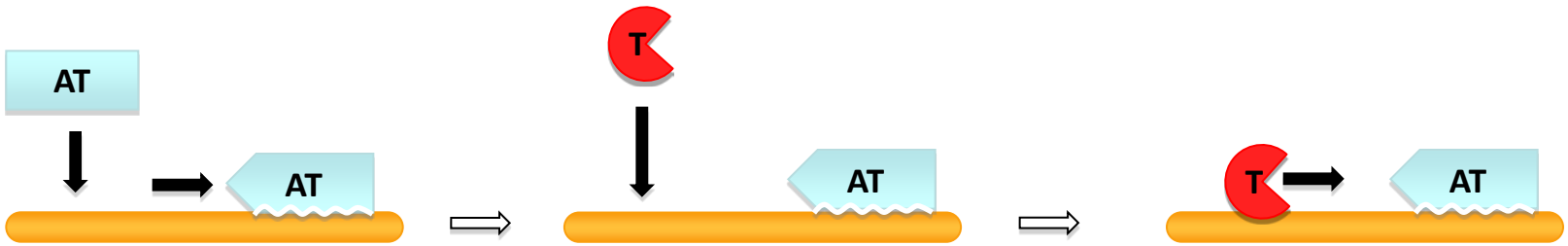
- **small molecule synthetic inhibitors**

heparin



- linear polysaccharide
- 5-40 kDa
- highly O and N sulfated and carboxylated
- biomacromolecule with highest (negative) charge density
- interaction with anion binding sites of proteins

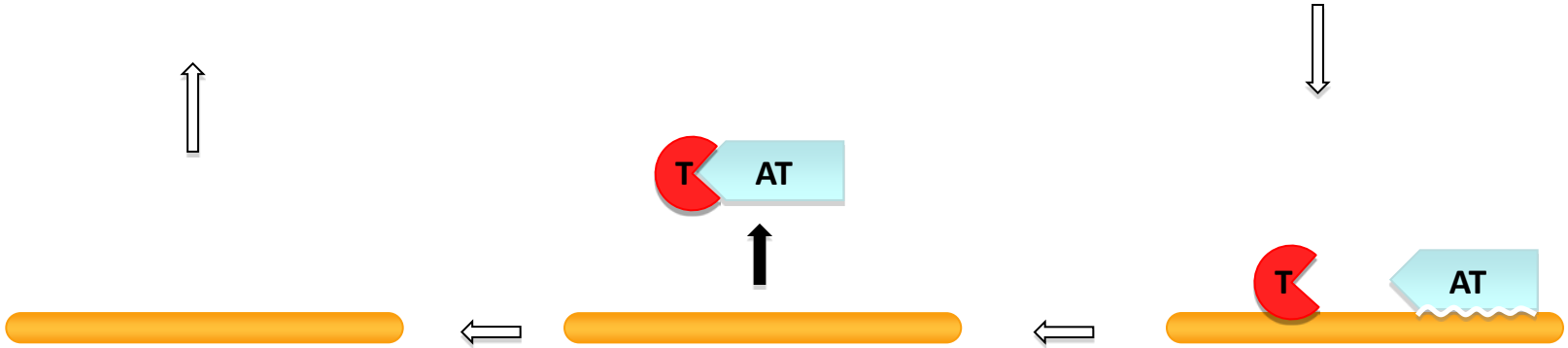
heparin – mode of action



- AT III adsorbs
- sticks at the pentasaccharide sequence

Thrombin adsorbs

Thrombin slides



Heparin can catalyse next inhibition cycle

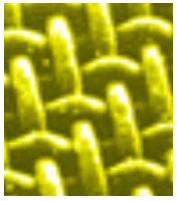
TAT complex dissociates, heparin „active sequence“ remains

Irreversible thrombin-antithrombin (TAT) complex assembles: coagulation effect of thrombin is neutralized

CBAS™ EN ISO 9001/EN 46001 CERTIFIED

carmeda bioactive surface works for

Vascular Grafts & Coronary Stents

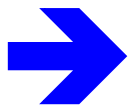


Cardiopulmonary Bypass Circuits
Ventricular Assist Devices

Central Venous Catheters
Intravascular Blood Gas Sensors

challenges of heparin coatings

- surface-bound heparin leaches
- low stability upon sterilization and *in vivo*…
- dependence on antithrombin
- limited safety (animal product)
- risk of HIT-II (heparin induced thrombocytopenia)



robust synthetic molecules
mimicking inhibitor functions

thrombin inhibition

coatings based on natural substances

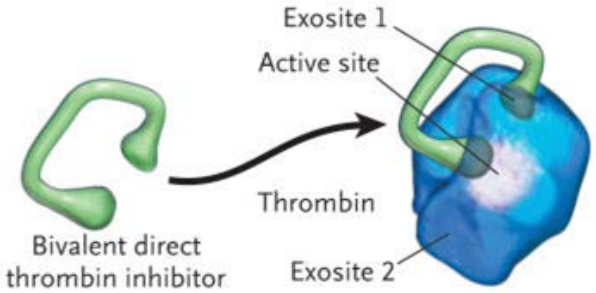
- **thrombomodulin** (complex formation with thrombin)
- **heparin** (activation of antithrombin AT)
- **hirudin** (direct thrombin inhibition)

coatings based on synthetic inhibitors

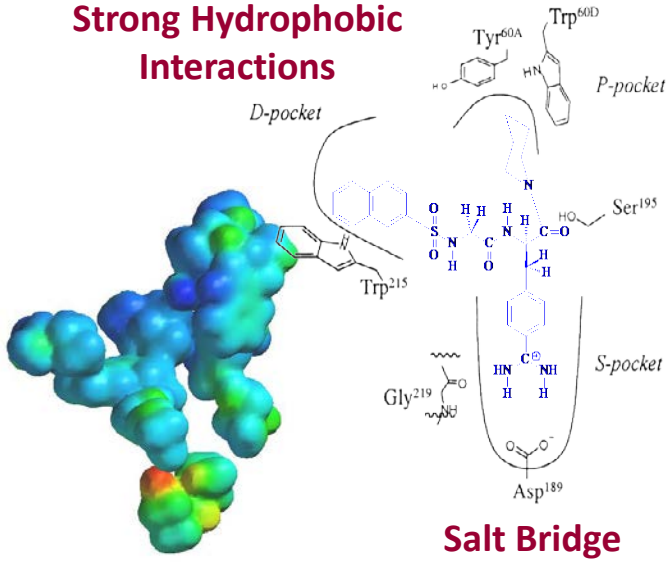
- **small molecule synthetic inhibitors**

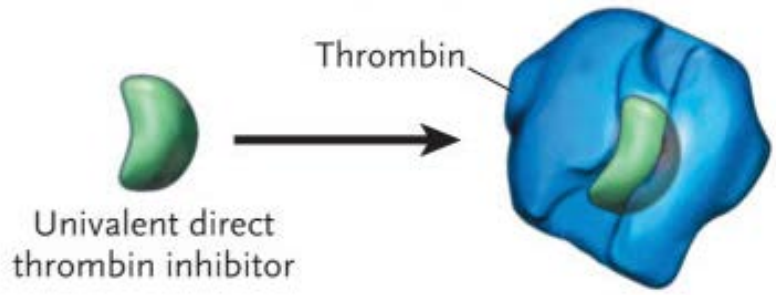
Hirudin inhibits **thrombin**, the key enzyme of the blood coagulation cascade

hirudo medicinalis



Synthetic inhibitors, e.g. NAPAP, can mimic this function





C. Lin, M. Tseng, Surface characterization and platelet adhesion studies on polyethylene surface with hirudin immobilization, Journal of Materials Science: Materials in Medicine 12 (2001) 827-832.

Challenges of bioactive protein immobilization

- high expenses
- immobilization without loss of activity
- stability upon shelf storage, sterilization, *in vivo* degradation
- safety (source of the product)

Look for more stable structures

thrombin inhibition

coatings based on natural substances

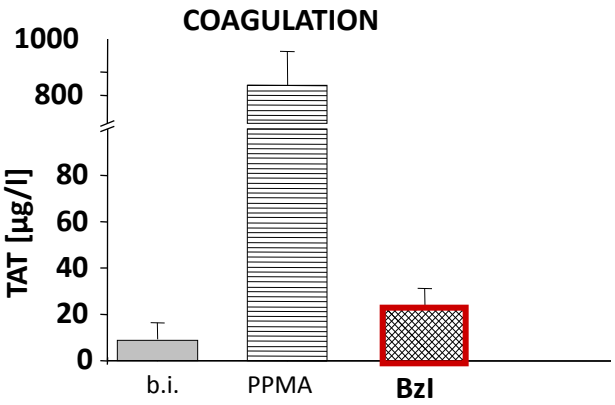
- **thrombomodulin** (complex formation with thrombin)
- **heparin** (activation of antithrombin AT)
- **hirudin** (direct thrombin inhibition)

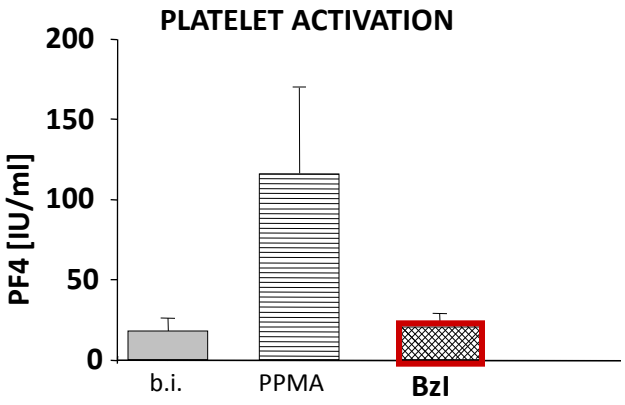
coatings based on synthetic inhibitors

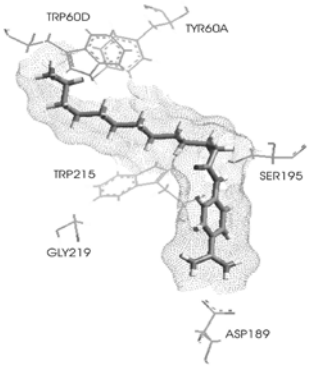
- **small molecule synthetic inhibitors**

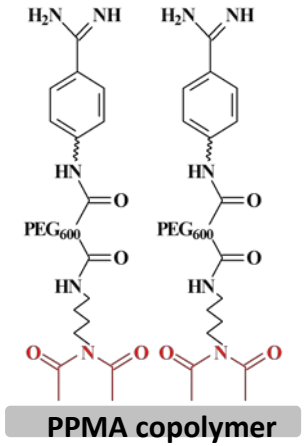
Covalently immobilized benzamidine layers minimize coagulation

Activation parameters after incubation in human whole blood (2 h)







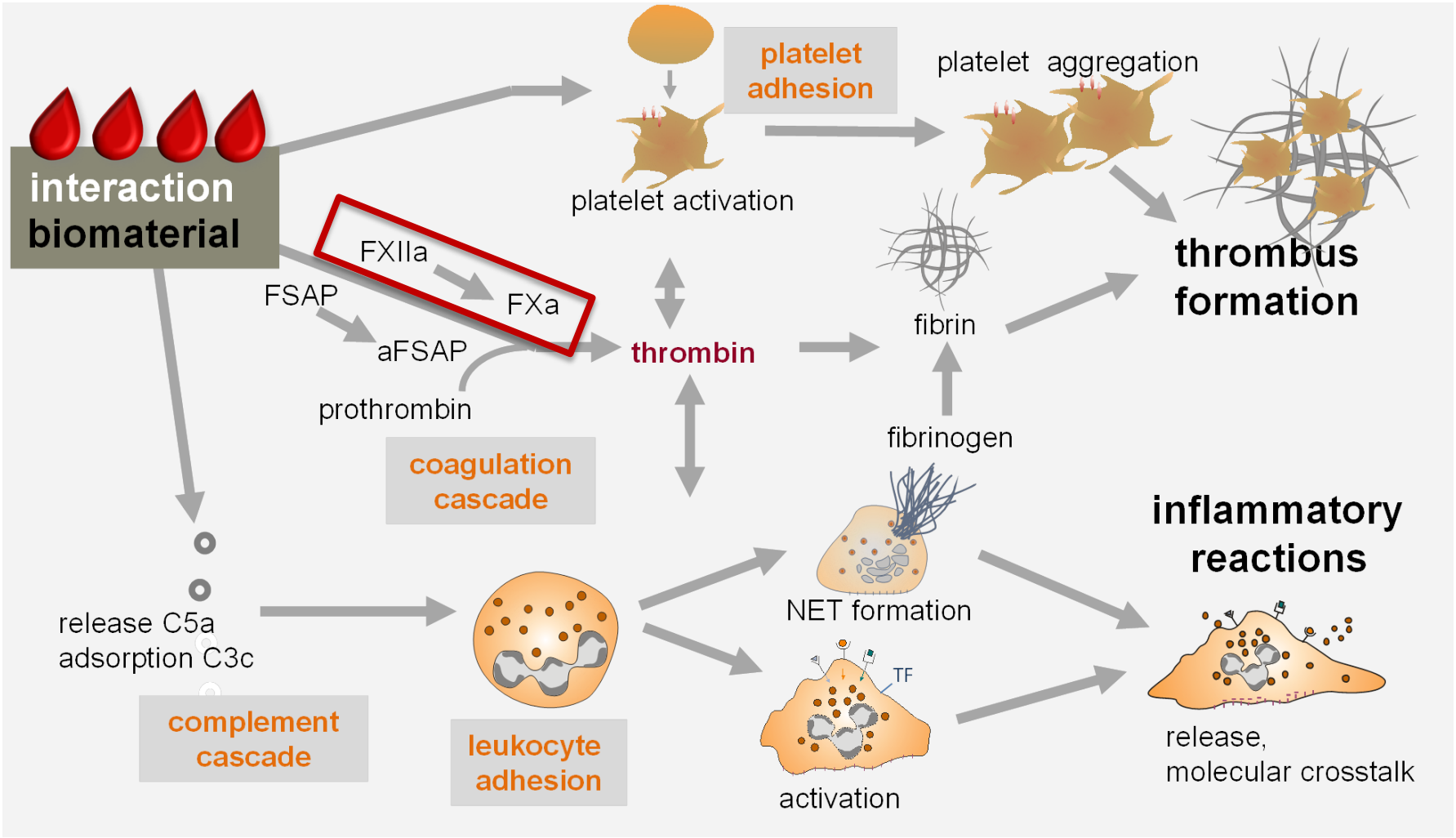


PPMA copolymer

BzI = PPMA + PEG spacer + **benzamidine**

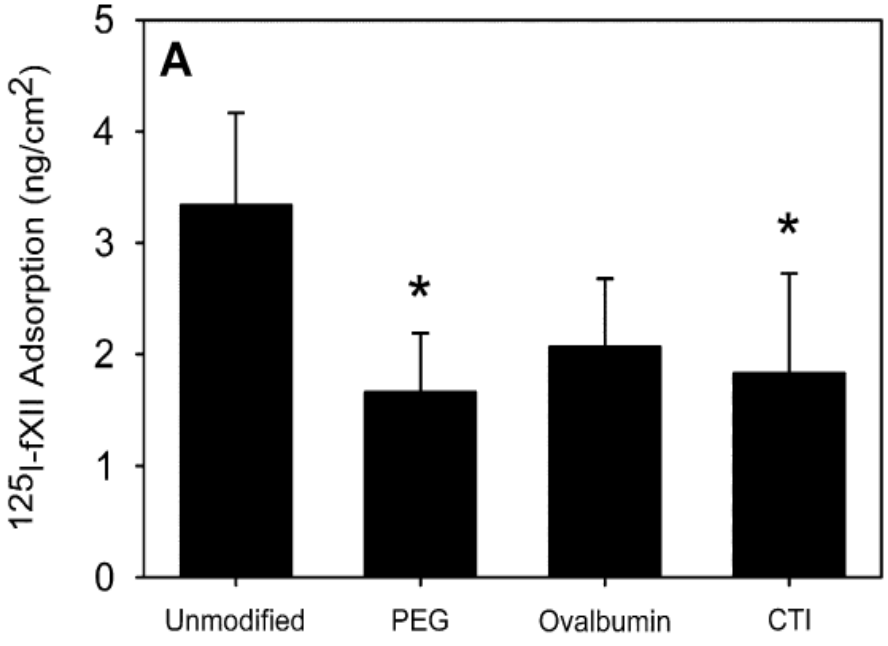
DE 103 30 900; DE 103 29 296; Biointerphases 2006, **1**, 1559; Acta Biomaterialia 2005, **1**, 441; Biomaterials 2005, **26**, 6547

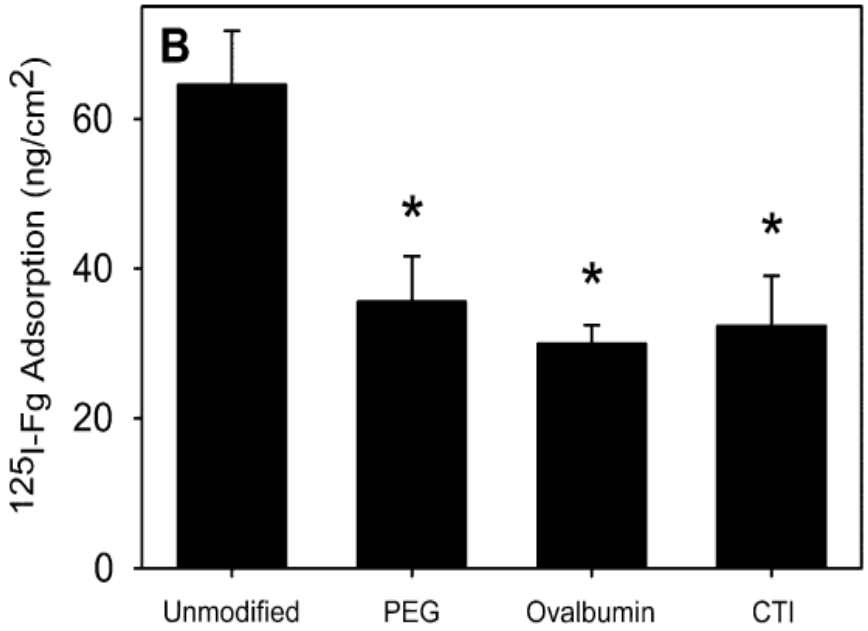
inhibition sites



inhibition of contact activation

immobilization of corn trypsin inhibitor

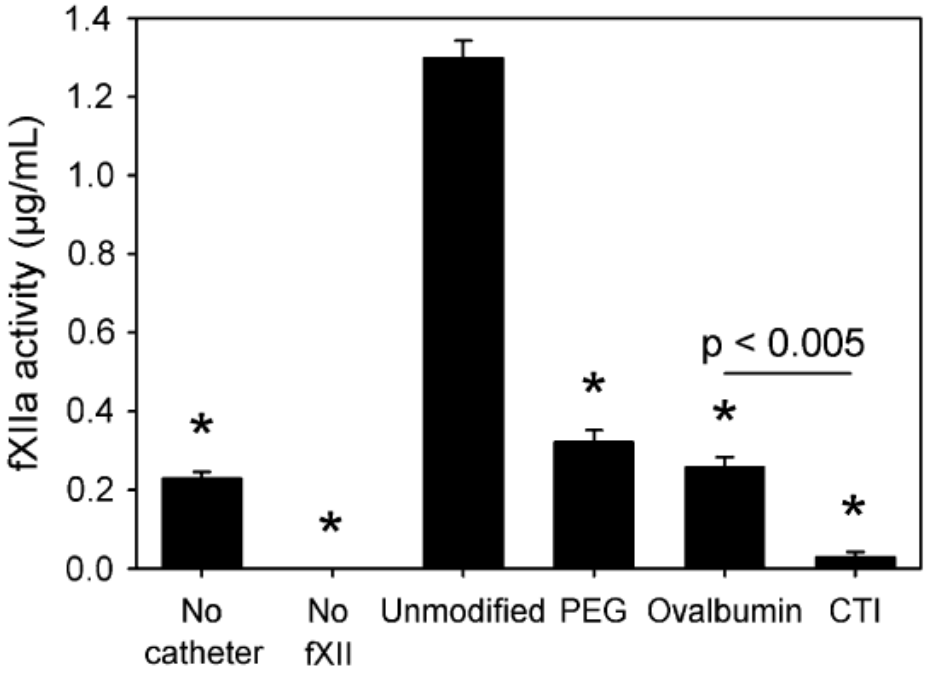


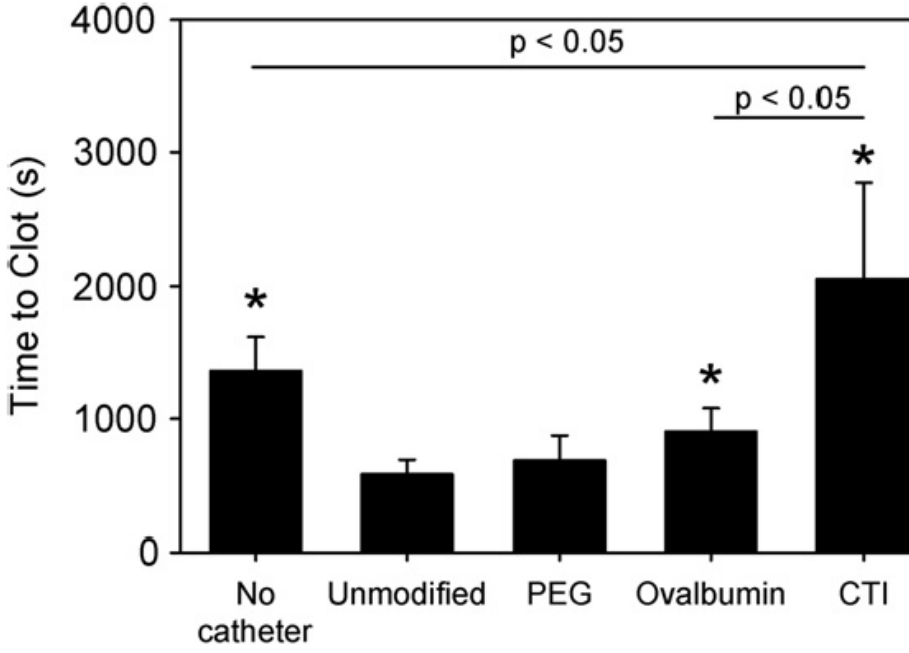


adsorption of ^{125}I- fXII or fibrinogen onto unmodified or modified PCI catheters in plasma.

J.W. Yau, A.R. Stafford, P. Liao, J.C. Fredenburgh, R. Roberts, J.L. Brash, J.I. Weitz, Corn trypsin inhibitor coating attenuates the prothrombotic properties of catheters in vitro and in vivo, Acta Biomaterialia 8(11) (2012) 4092-100.

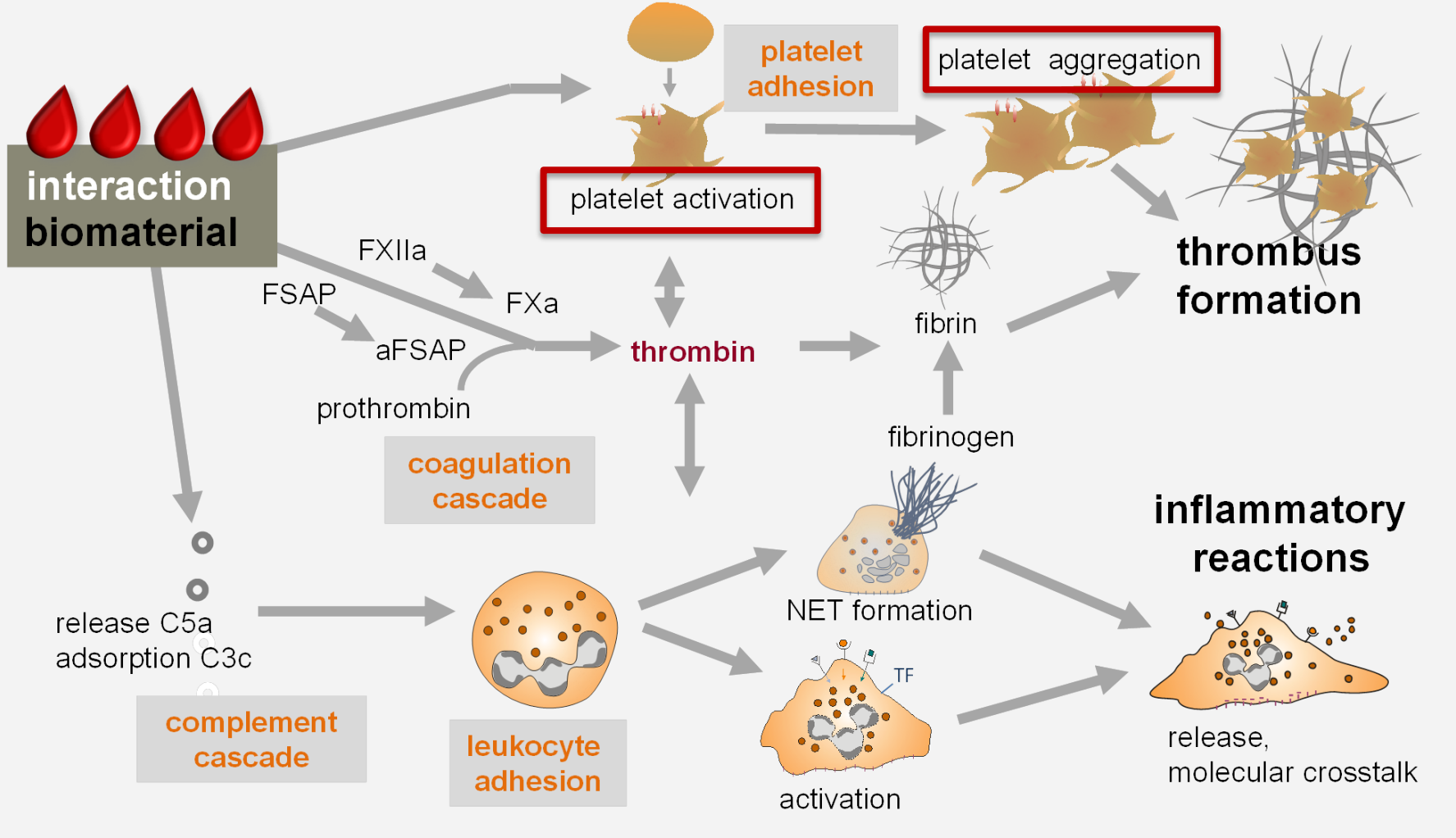
inhibition of contact activation





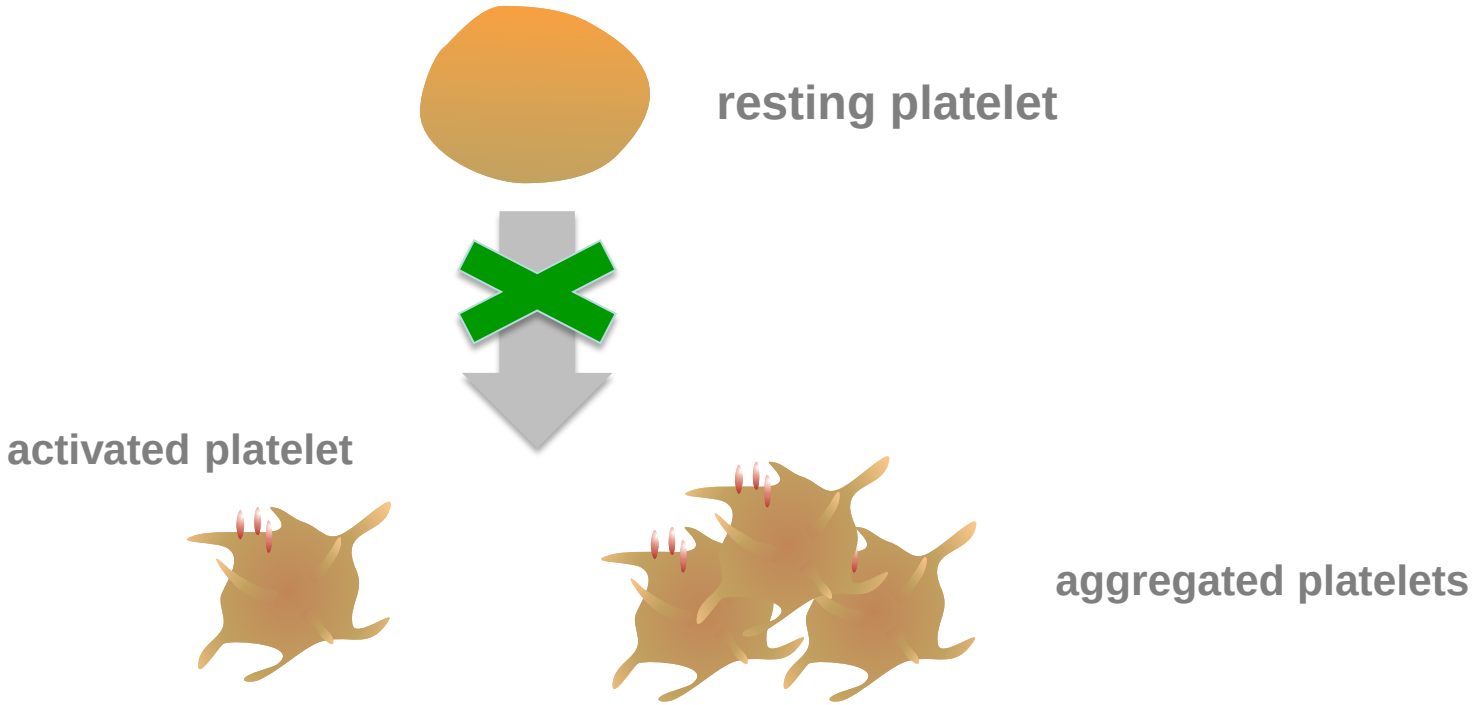
J.W. Yau, A.R. Stafford, P. Liao, J.C. Fredenburgh, R. Roberts, J.L. Brash, J.I. Weitz, Corn trypsin inhibitor coating attenuates the prothrombotic properties of catheters in vitro and in vivo, Acta Biomaterialia 8(11) (2012) 4092-100.

inhibition sites



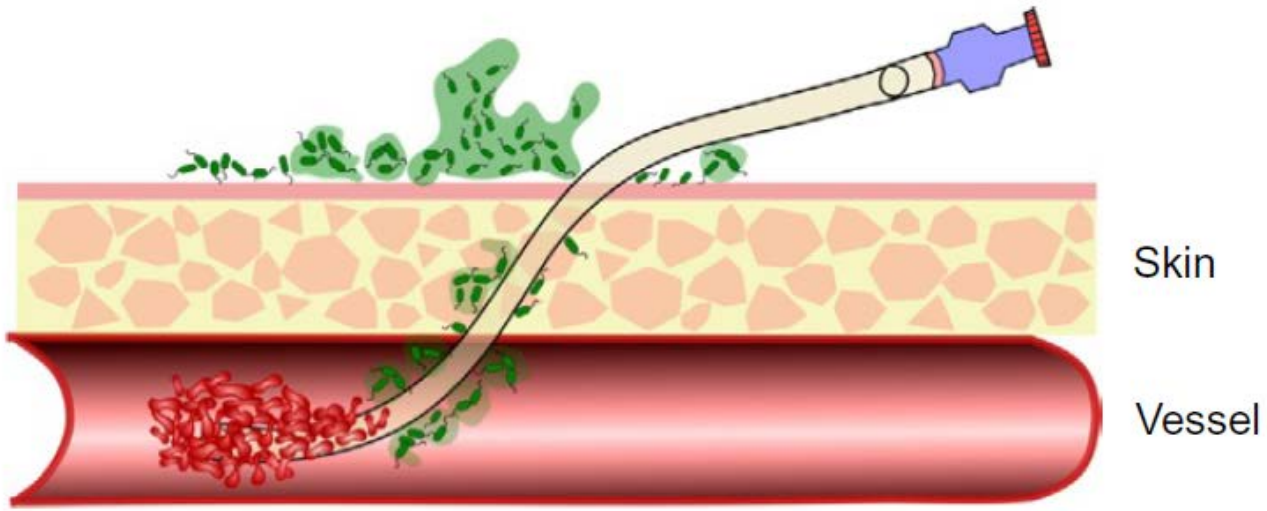
inhibition of platelet activation and aggregation

- dipyridamol
- prostaglandin PGE1
- nitric oxide



Improving the hemocompatibility of catheters via NO release/generation

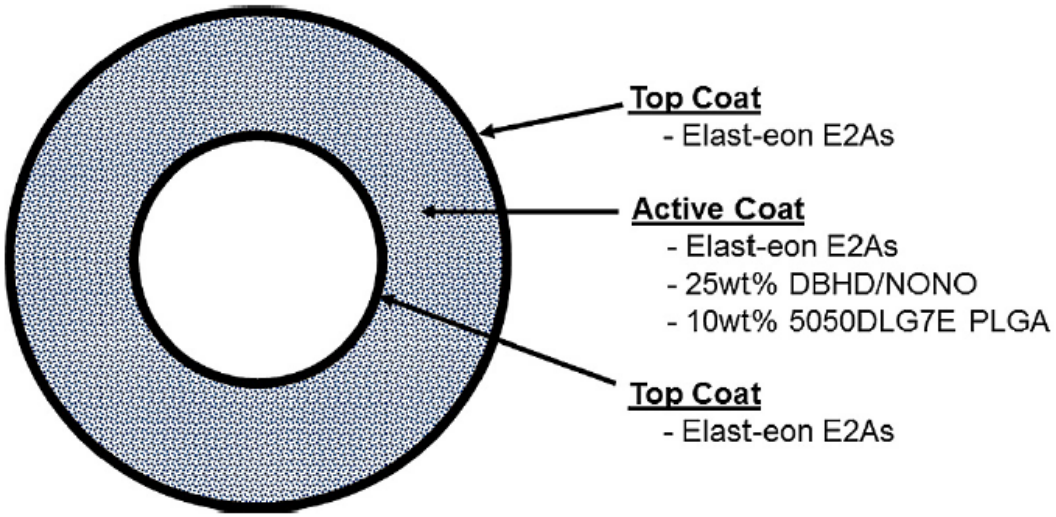
Y. Wo, E.J. Brisbois, R.H. Bartlett, M.E. Meyerhoff
University of Michigan, Ann Arbor, MI, United States



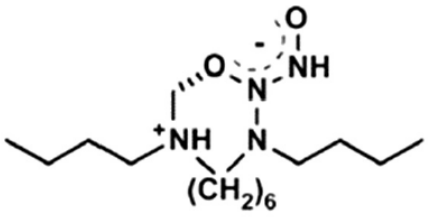
thrombus formation (*red*) and bacterial infection (*green*) on surface of intravascular catheters

nitric oxide releasing surfaces

NO: versatile regulator: inhibition of SMCs, platelets and leukocyte chemotaxis, antibacterial

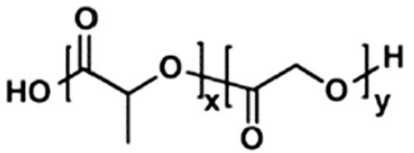


A



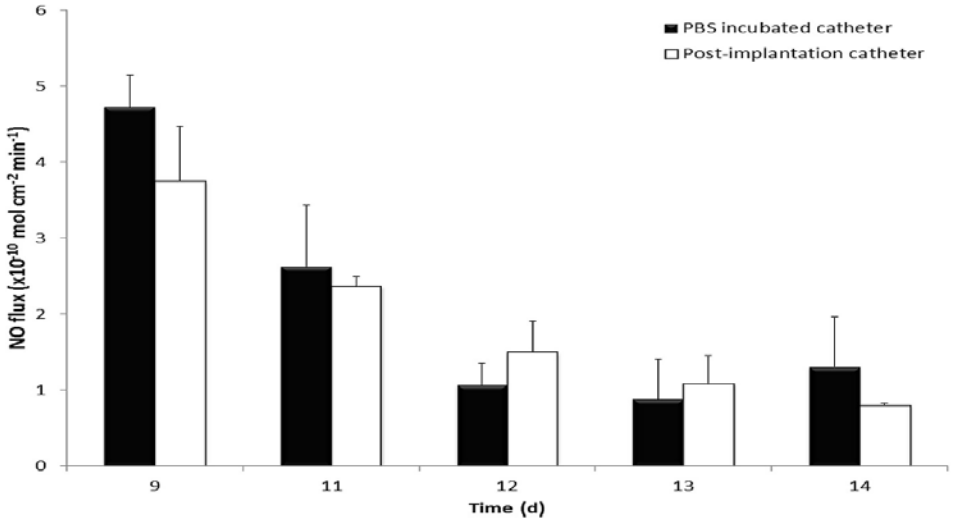
Diazeniumdiolated dibutylhexanediamine
DBHD/NONO

B

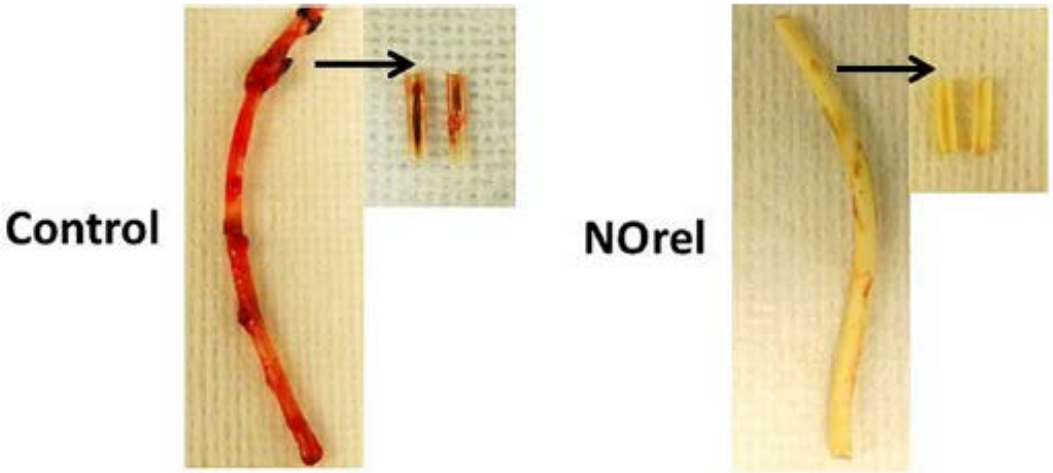


Poly(lactide-*co*-glycolide)
PLGA

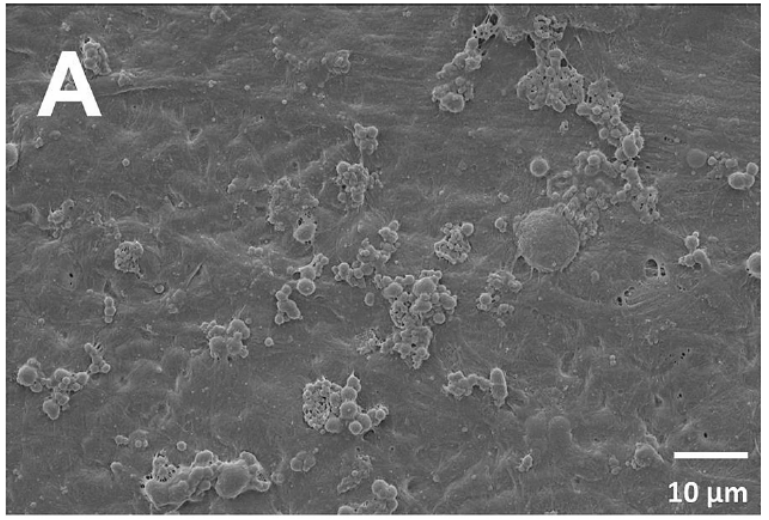
E.J. Brisbois, T.C. Major, M.J. Goudie, M.E. Meyerhoff, R.H. Bartlett, H. Handa, Attenuation of thrombosis and bacterial infection using dual function nitric oxide releasing central venous catheters in a 9 day rabbit model, Acta Biomaterialia 44 (2016) 304-312.

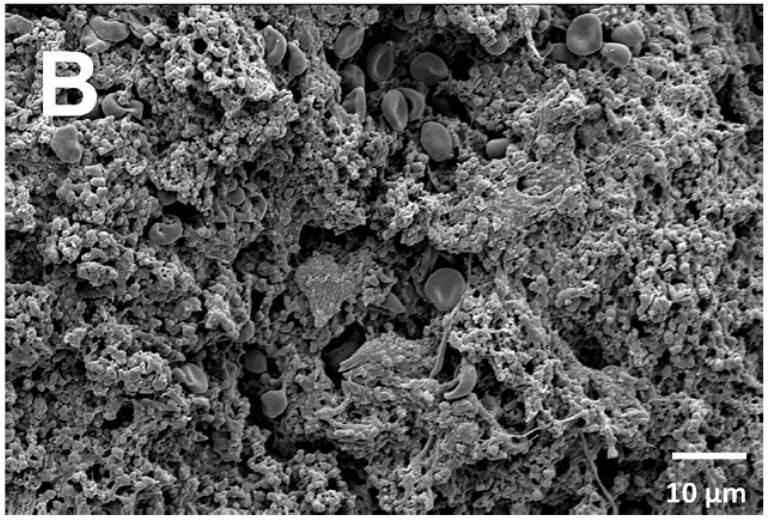


NO flux



implantation in rabbit veins for 9 days





(A) NO-releasing
(B) control catheters

E.J. Brisbois, T.C. Major, M.J. Goudie, M.E. Meyerhoff, R.H. Bartlett, H. Handa, Attenuation of thrombosis and bacterial infection using dual function nitric oxide releasing central venous catheters in a 9 day rabbit model, Acta Biomaterialia 44 (2016) 304-312.

nitric oxide releasing surfaces

NO donor substrates

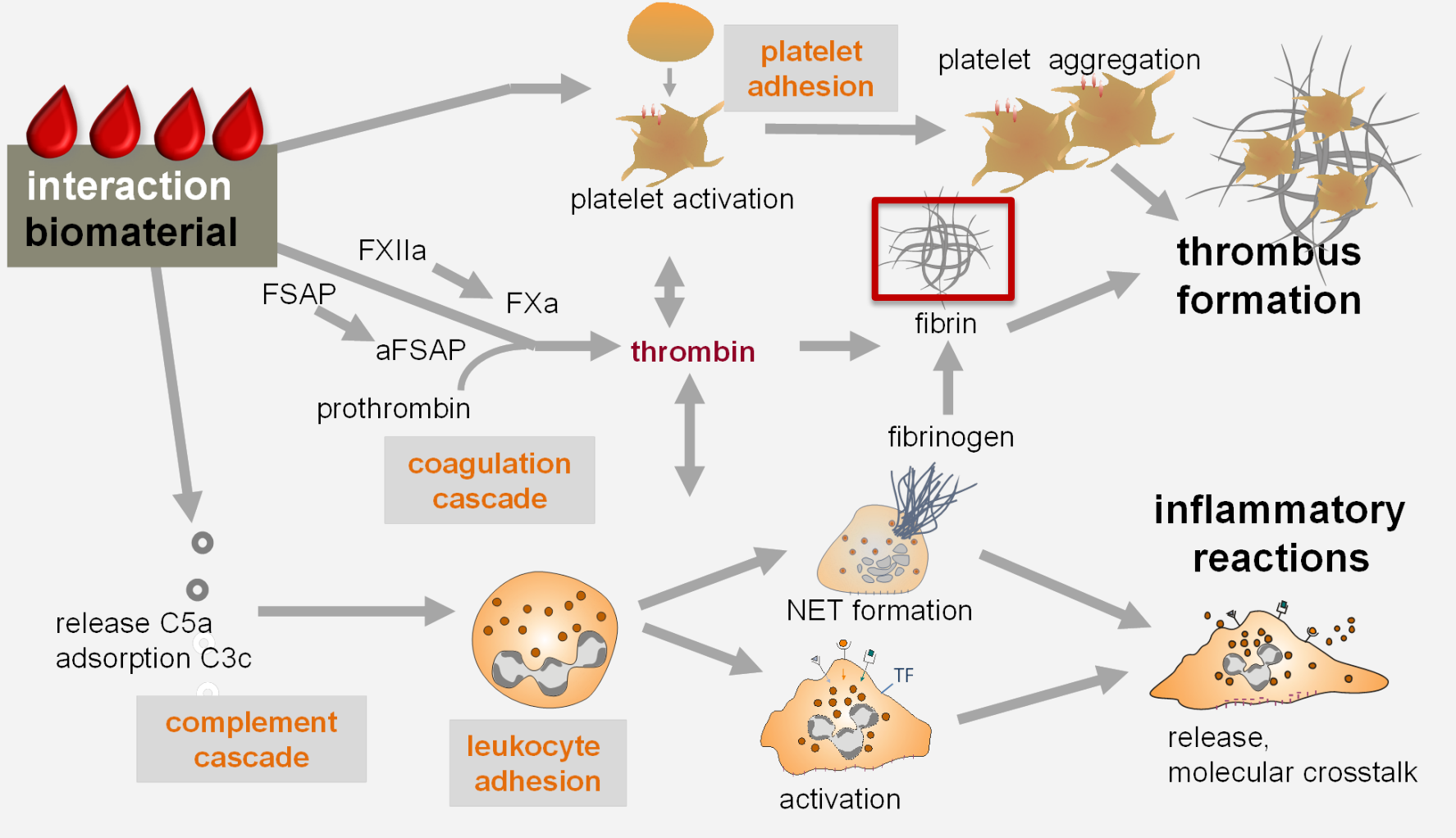
- limited amount released
- release of carcinogenic nitrosamines

catalytic agents: agents capable of synthesizing NO using physiologic sources

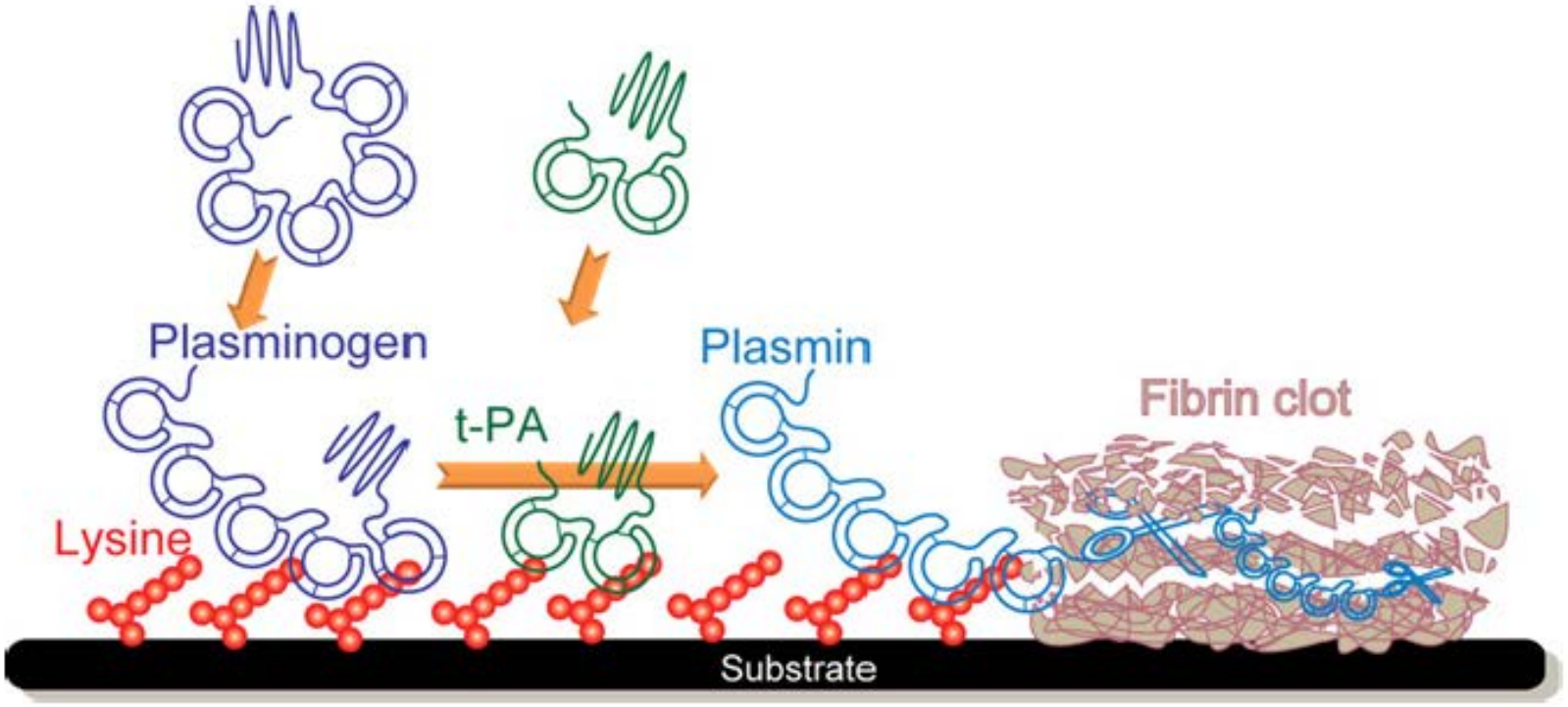
- cystein modified polymers
- doping of surfaces with Cu^{+}

Clinical validation not yet successful

inhibition sites



activation of fibrinolysis



Schematic representation of the lysine-based clot lysing surface plasmin degrades insoluble fibrin clots to generate soluble fibrin fragments

D. Li, H. Chen, J.L. Brash, Mimicking the fibrinolytic system on material surfaces, Colloids and Surfaces B: Biointerfaces 86(1) (2011) 1-6.

activation of fibrinolysis

surface modification substances

direct immobilization

- plasminogen activators (t-PA, u-PA)
- recombinant compounds (alteplase, reteplase, …)

lysine-functionalization

- captures plasmatic t-PA

fast reaction is necessary: detachment of complete thrombus – embolization possible!

no successful clinical trials yet

D. Li, H. Chen, J.L. Brash, Mimicking the fibrinolytic system on material surfaces, Colloids and Surfaces B: Biointerfaces 86(1) (2011) 1-6.

challenges of direct inhibitors

- dosage invariant and limited by surface area of device
- restricted accessibility of active component in layered material
- other activation pathways remain active

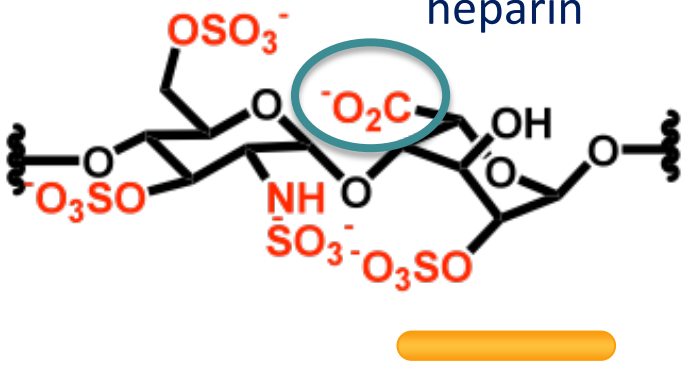
Surface functionalization for hemocompatibility

passivation - active inhibition
permanent - renewable

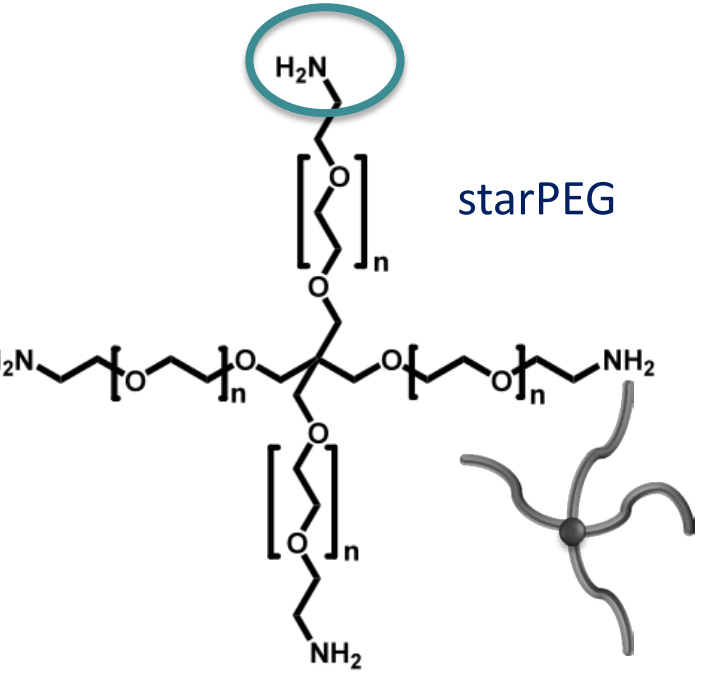
Modular polymer networks

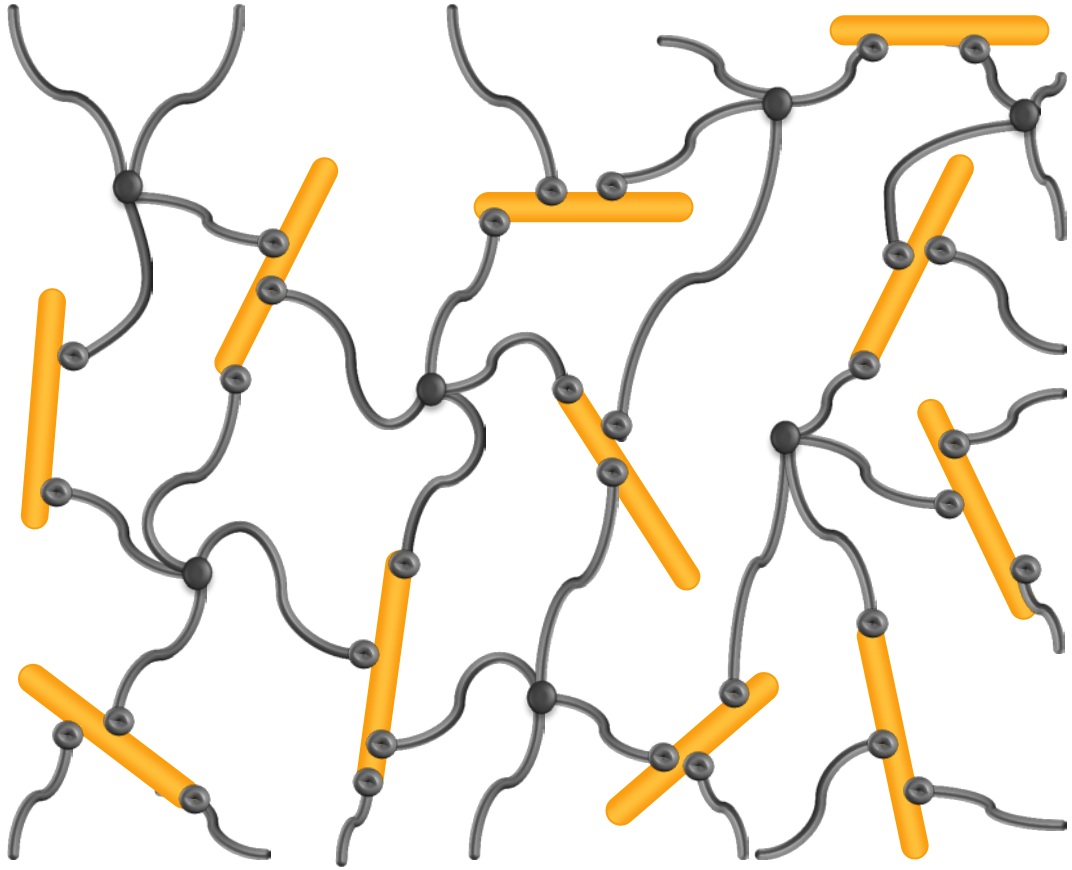
based on heparin and 4-armed, end-functionalized polyethylene glycol (starPEG)…

heparin



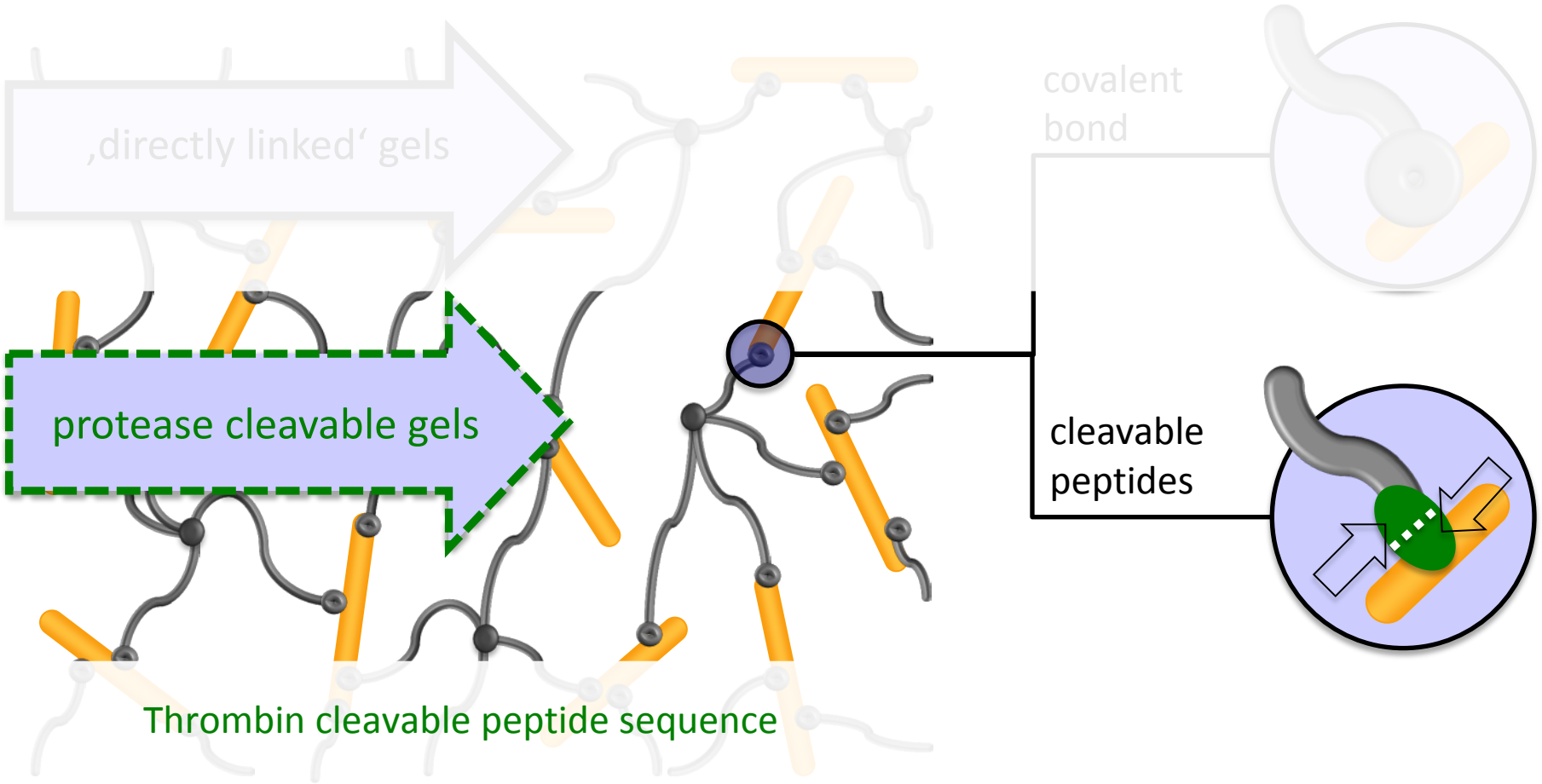
starPEG



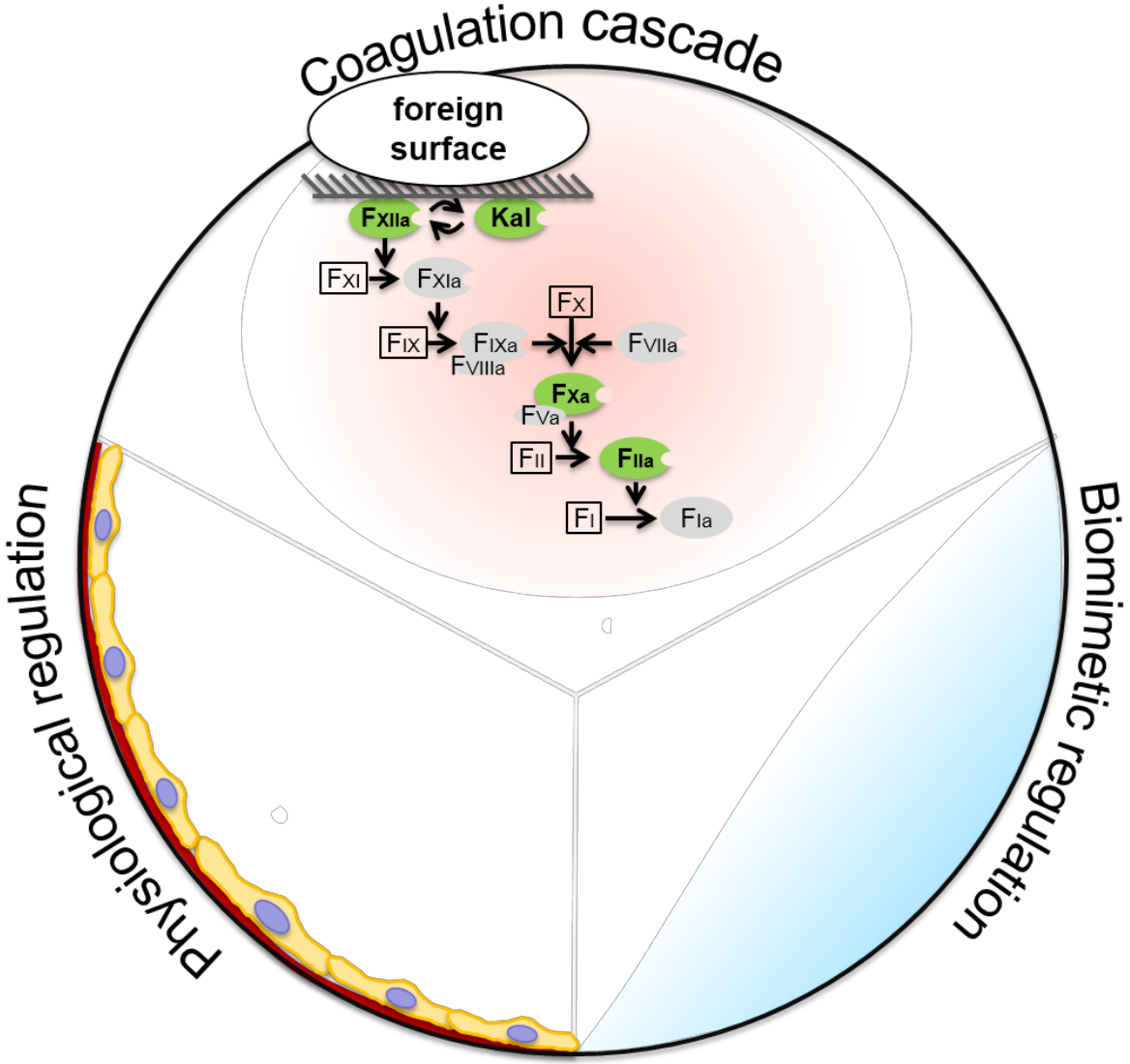


Crosslinking principles of the hydrogel

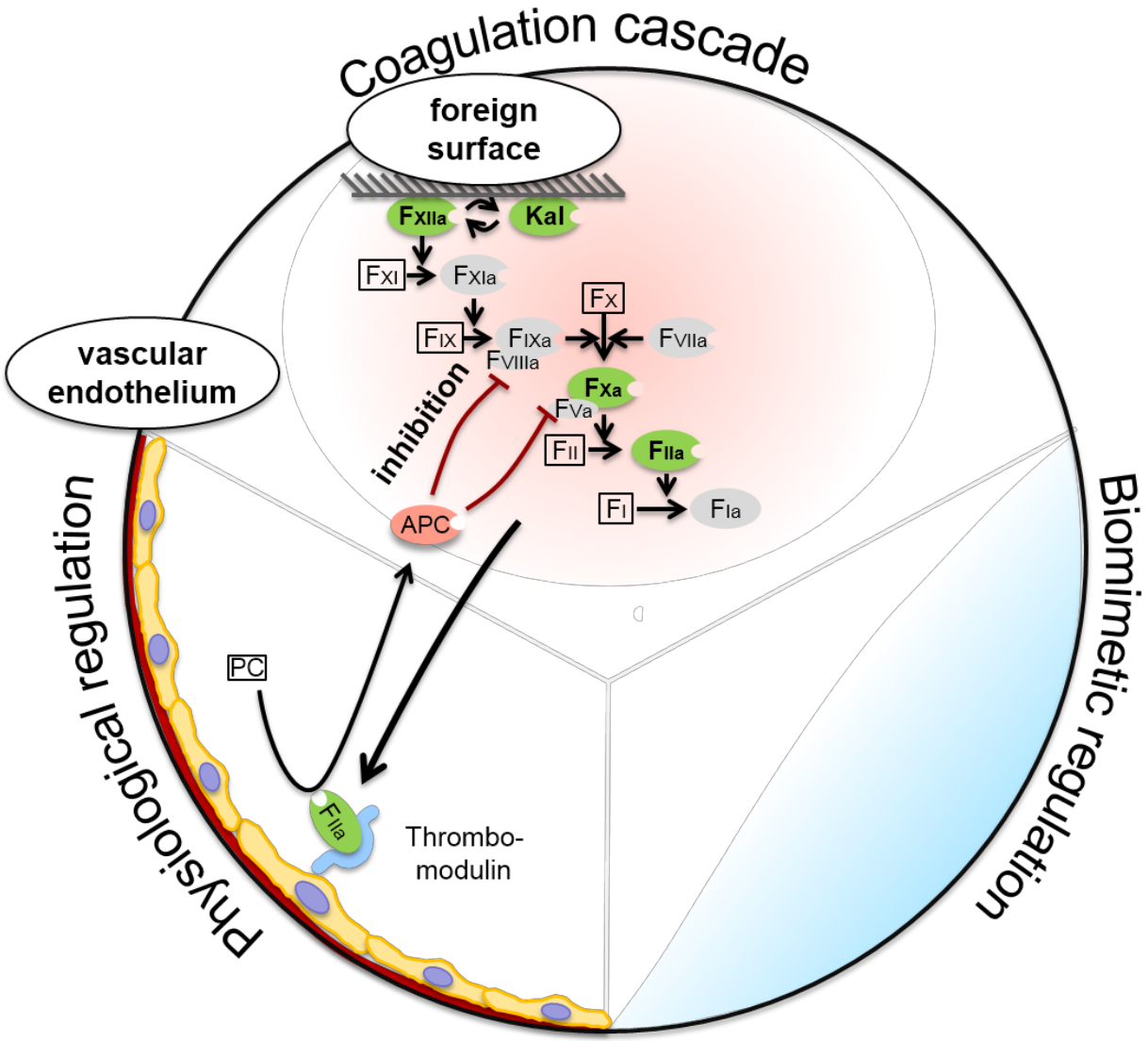
(with/without incorporation of peptides)



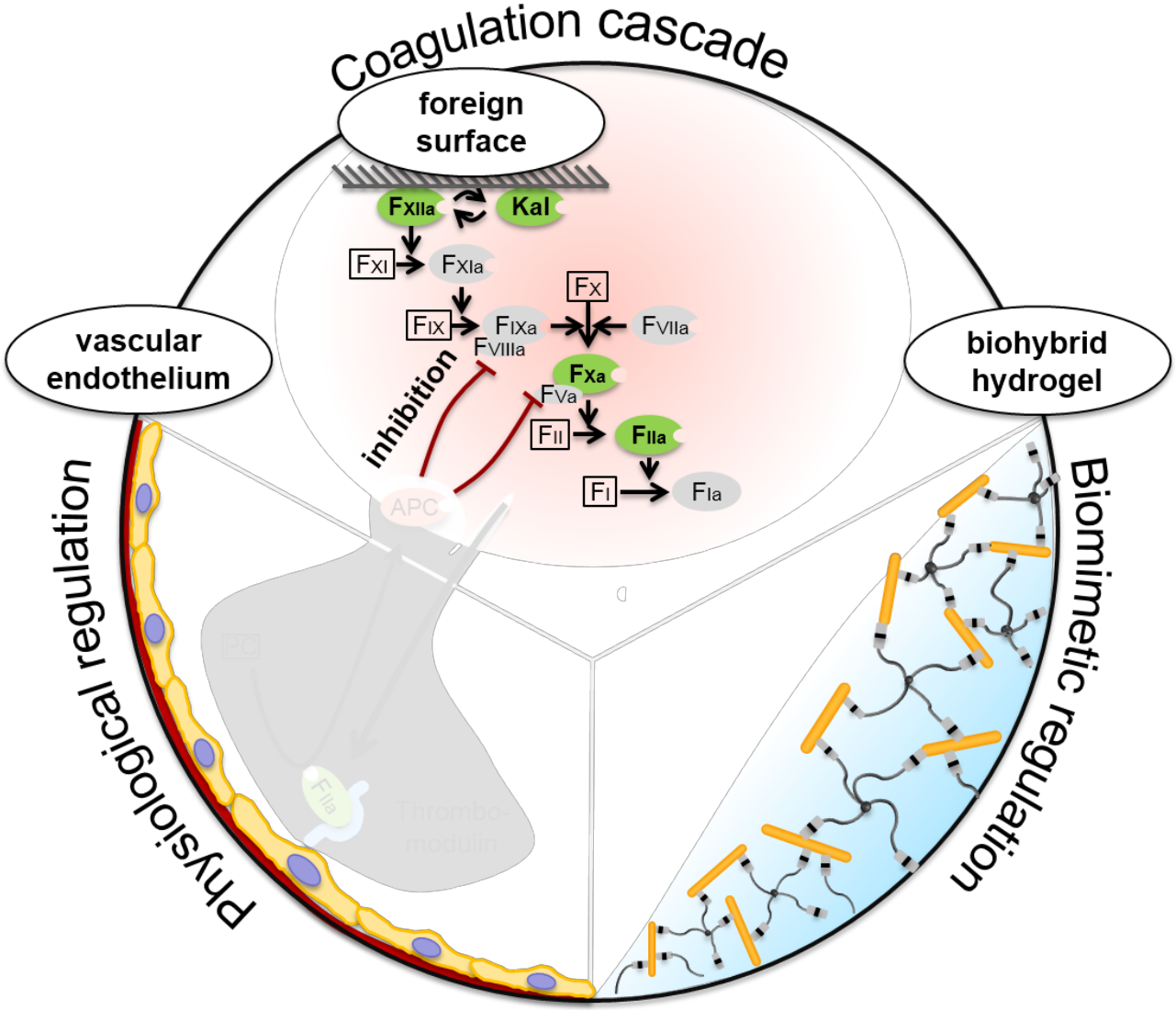
Similarity with endothelial cogulation control



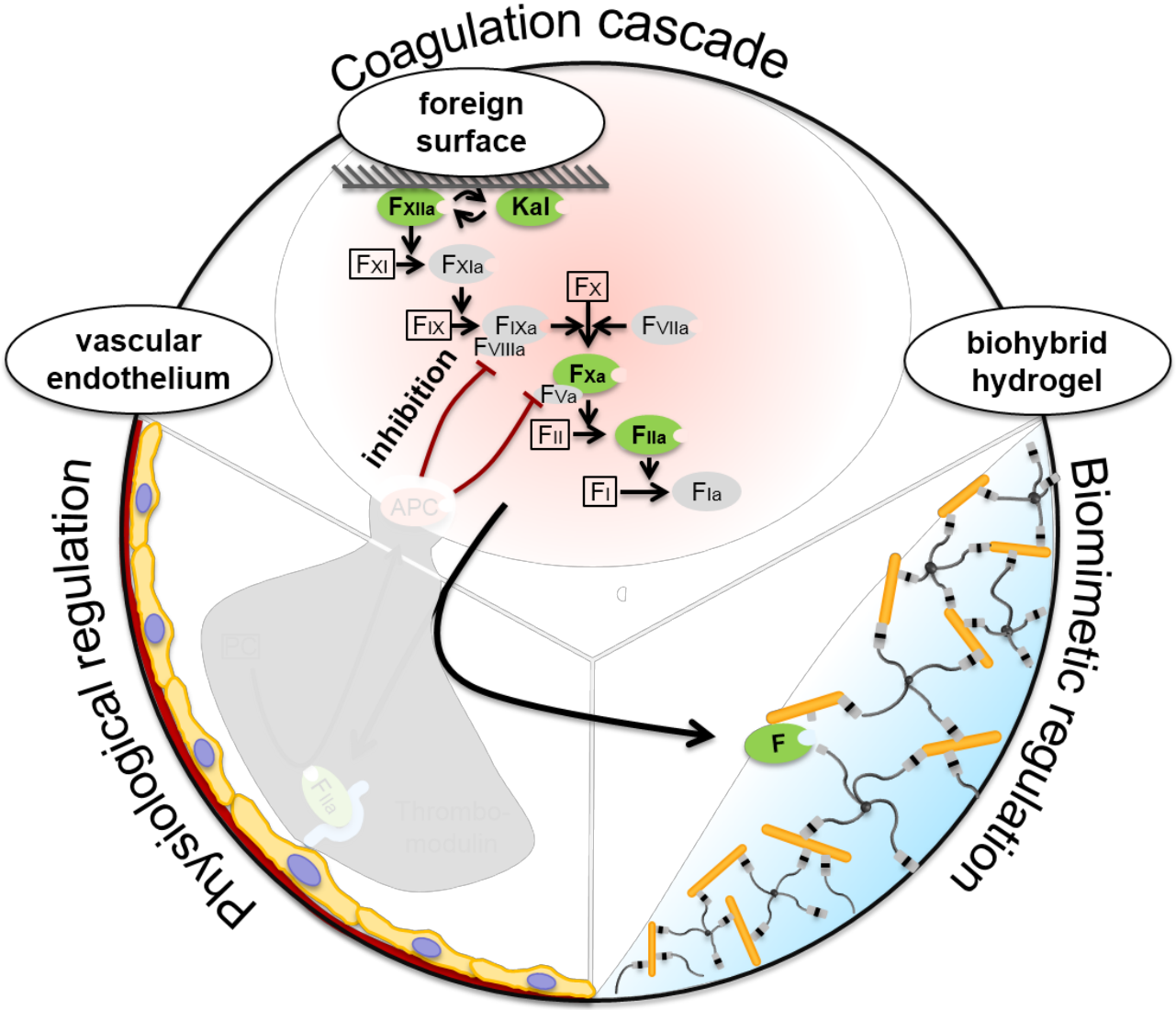
Similarity with endothelial cogulation control



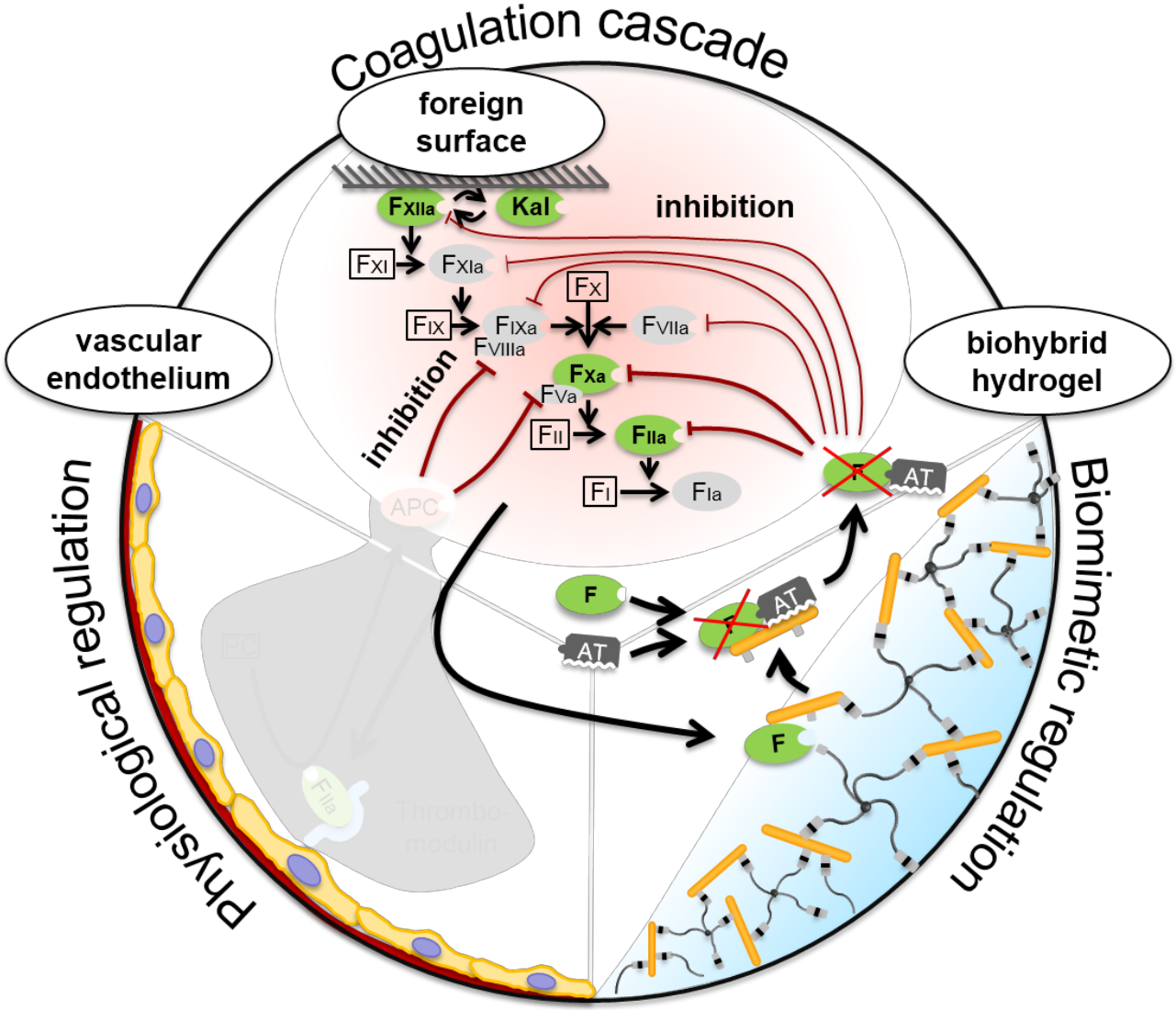
Similarity with endothelial cogulation control



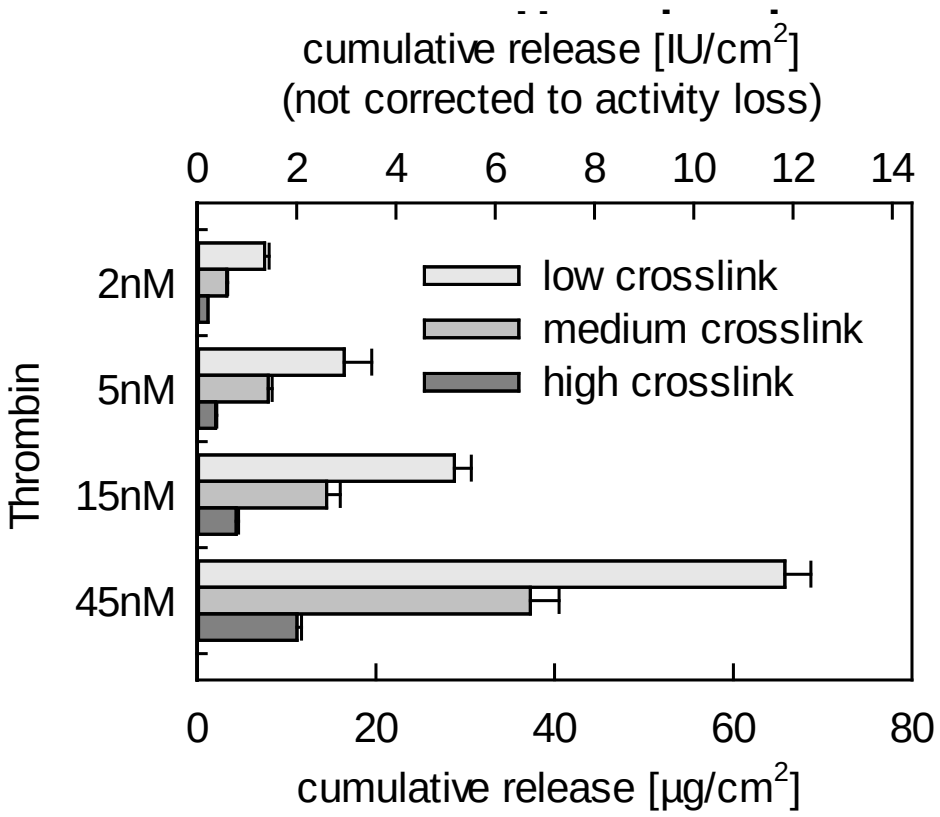
Similarity with endothelial cogulation control



Similarity with endothelial cogulation control

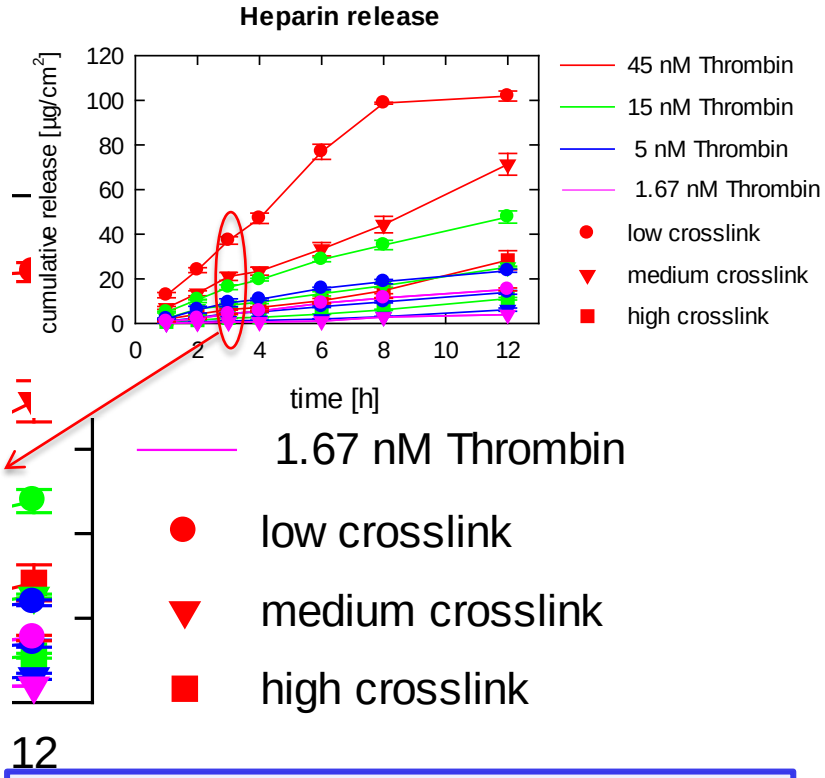


Gel degradation in thrombin solution



Variation of

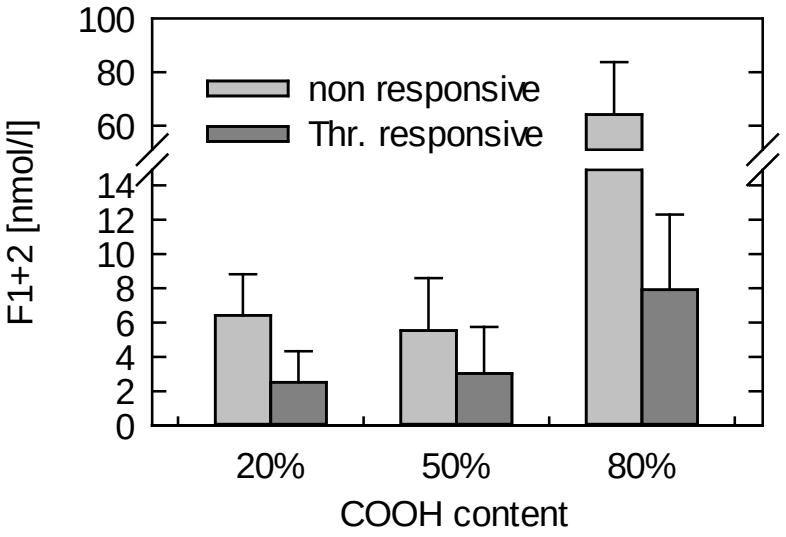
- Crosslinking degree
- Thrombin concentration

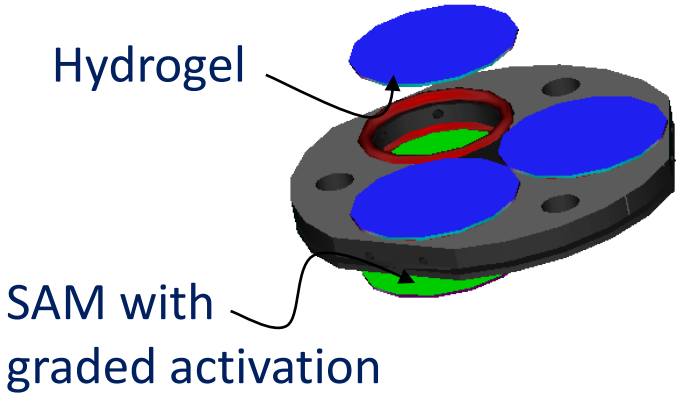


- linear release kinetics
- release scales with crosslinking degree and thrombin level

Coagulant stress-test

Whole blood incubation: co-incubation with pro- coagulant surfaces

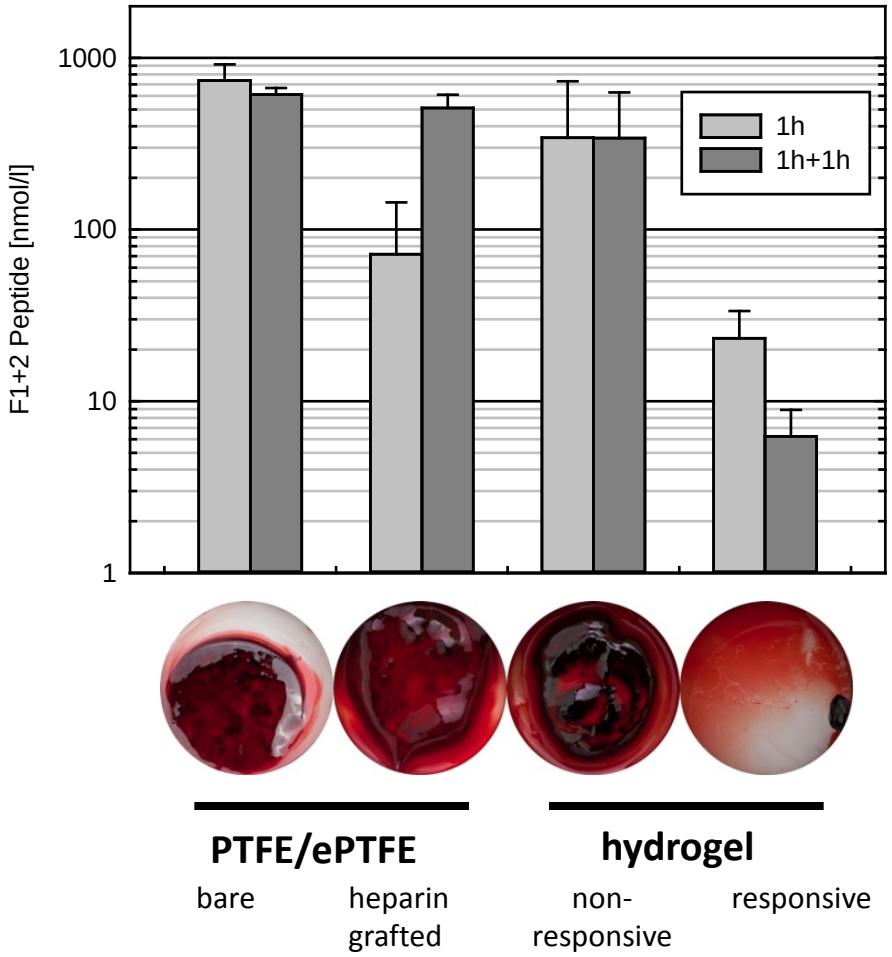




co-incubation
stable vs. thrombin-cleavable gels

Thrombin cleavable gels suppress coagulation of external activators

Incubation with non-anticoagulated whole blood



thrombin **responsive hydrogel outperforms references** including non-responsive gels and endpoint heparinized ePTFE

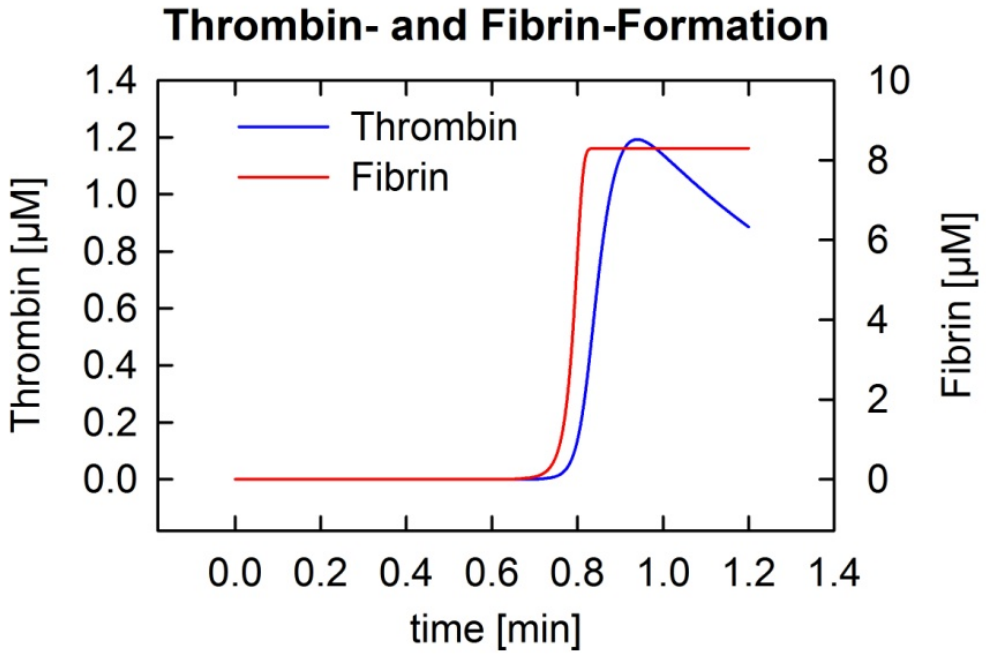
enhanced anticoagulant effect of responsive gel **upon repeated incubation** with fresh blood

only responsive hydrogel **prevents clotting**

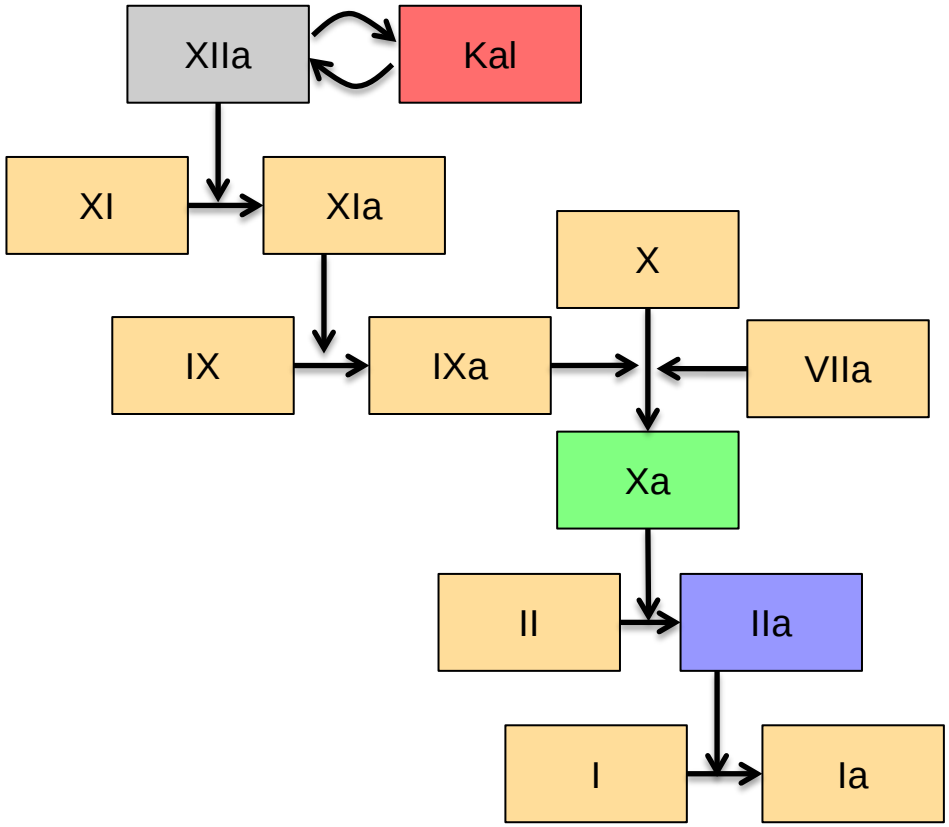
M.F. Maitz, U. Freudenberg, M.V. Tsurkan, M. Fischer, T. Beyrich, C. Werner, Bio-responsive polymer hydrogels homeostatically regulate blood coagulation, Nat Commun 4 (2013) 2168.

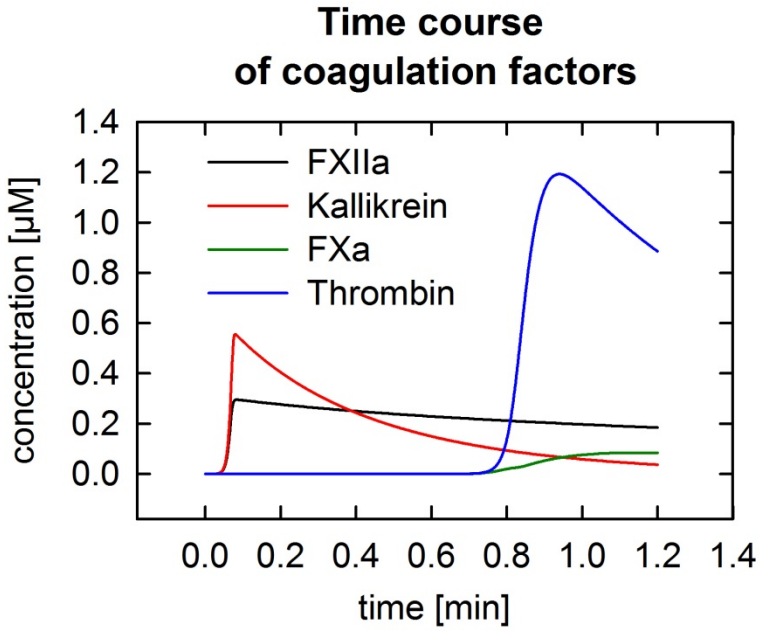
Thrombin as hydrogel-degrading protease

- High plasma concentration
  - High gel degradation promised
- High affinity to heparin and accumulation at the hydrogel
  - Increased activity?
- Very late protease in congulation process
  - Would earlier heparin release be more efficient?



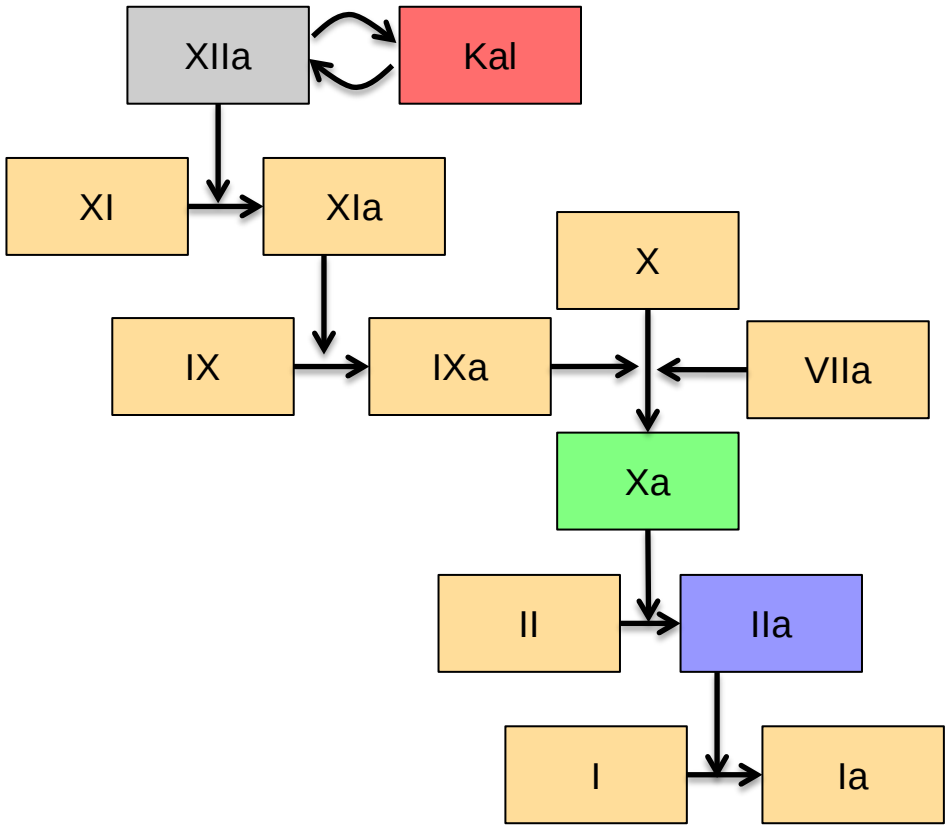
Different triggers for heparin release





Faster reaction by hydrogels with response to other coagulation factors

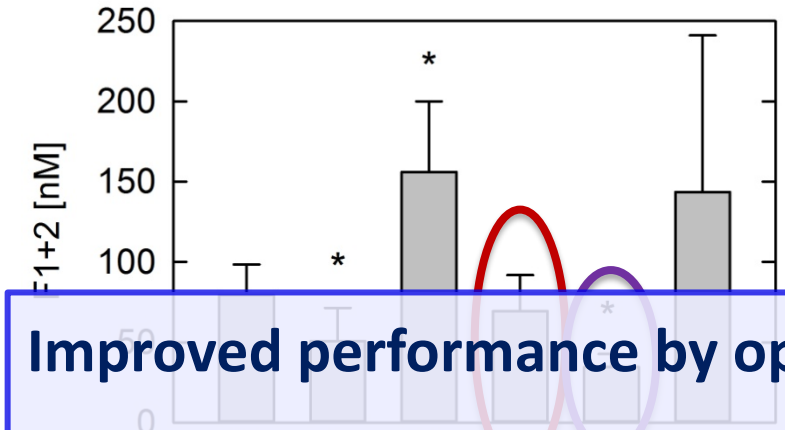
Different triggers for heparin release

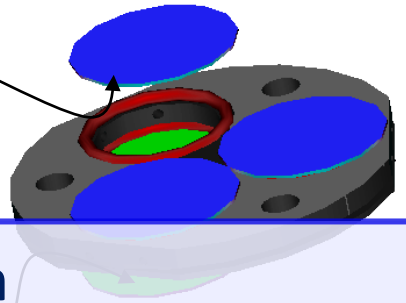


- FXIIa/Kallikrein
  - Contact phase activation
  - Very early activation
  - Low inhibition by heparin
- FXa
  - Low plasma concentration
  - In prothrombinase complex no heparin affinity
  - Peak concentration after thrombin
- Thrombin
  - High concentration
  - Heparin affinity
  - Rapid fibrin formation

Faster reaction by hydrogels with response to other coagulation factors

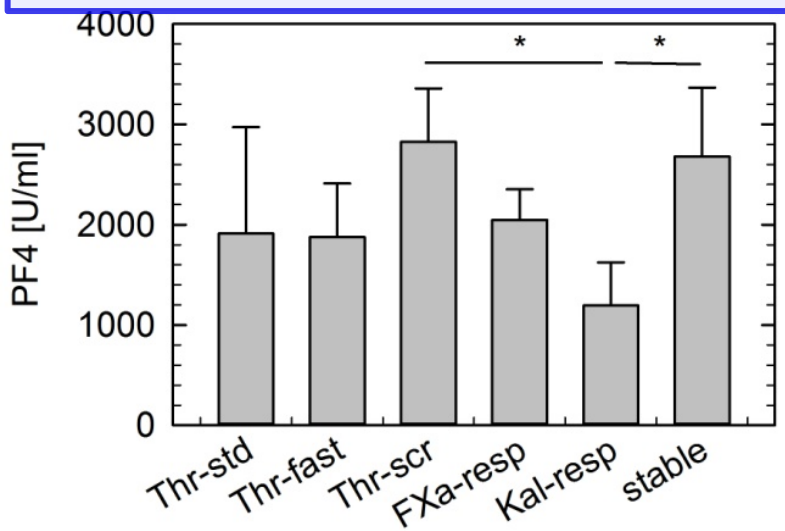
Whole blood incubation

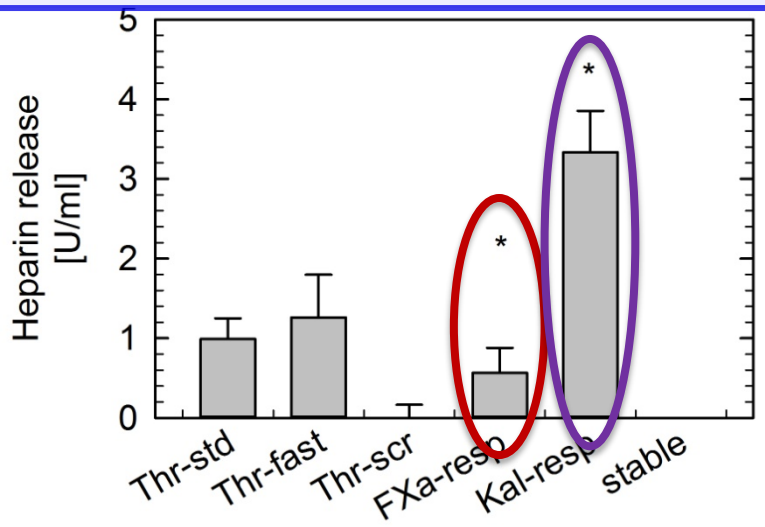




Improved performance by optimized design

Inhibitor release upon early coagulation factor FXa:
less drug release has same biological effect



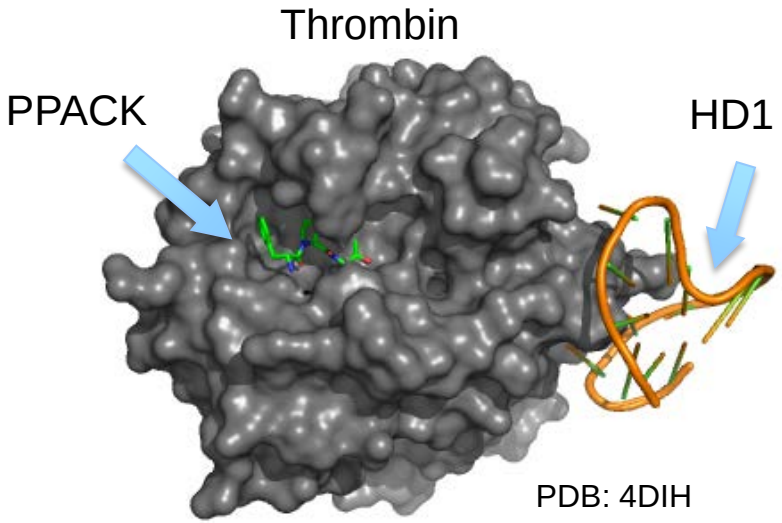


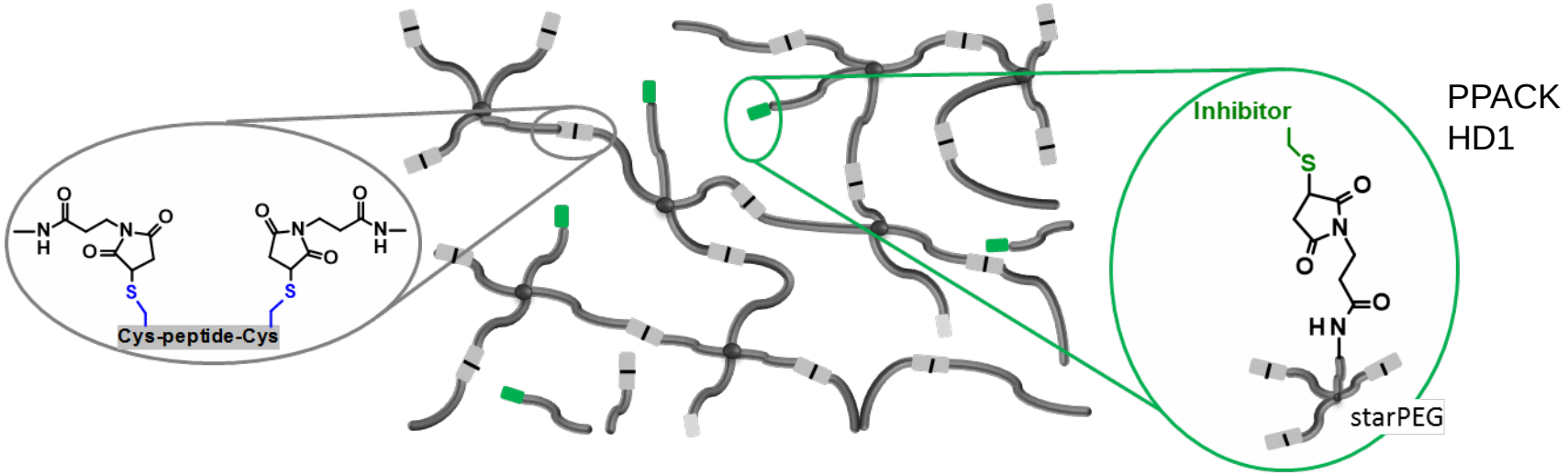
Limitations of heparin dependence

- side effects
- indirect anticoagulant: requires antithrombin
- Structure- and effective molecule in the hydrogel: Independent optimization not possible

Responsive PEG hydrogels for delivery of synthetic inhibitors

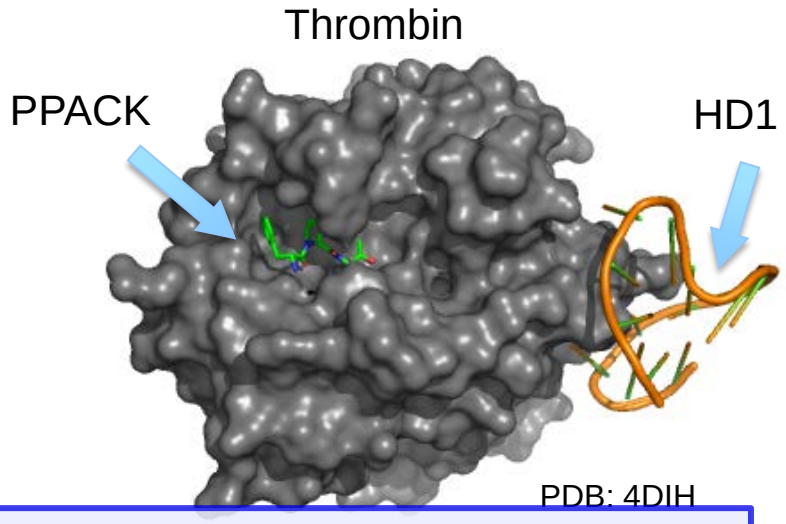
- PPACK (Peptide)
- HD1 (DNA-Aptamer)



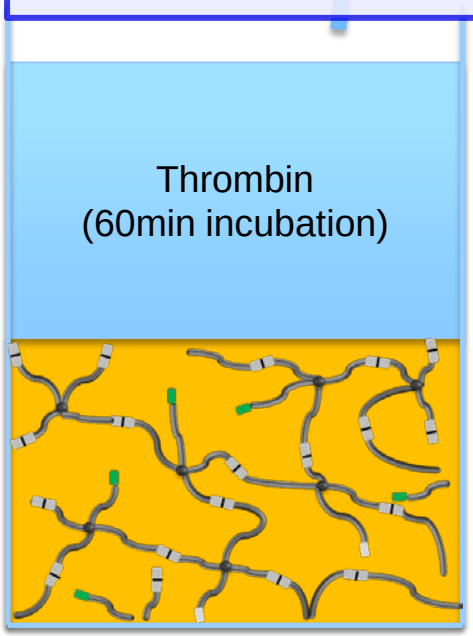


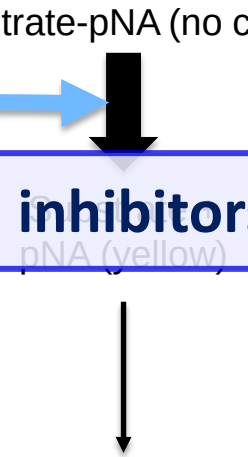
Responsive PEG hydrogels for delivery of synthetic inhibitors

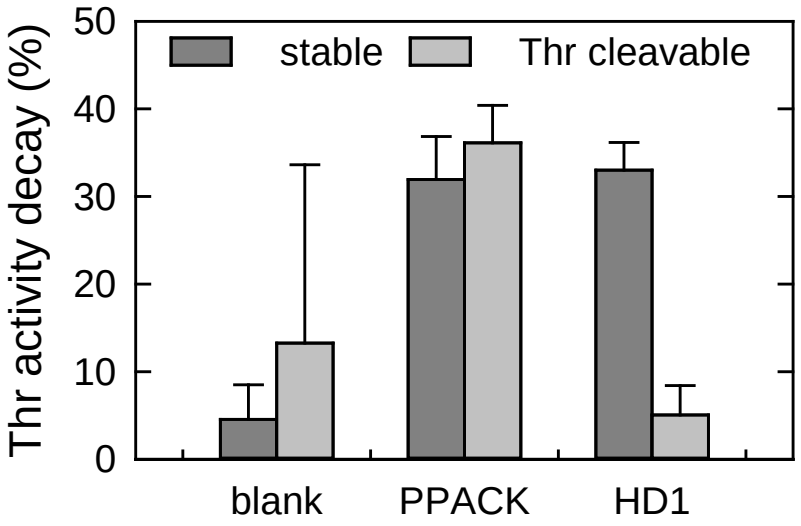
- Function in buffer



Direct thrombin inhibitors linked to hydrogels are active

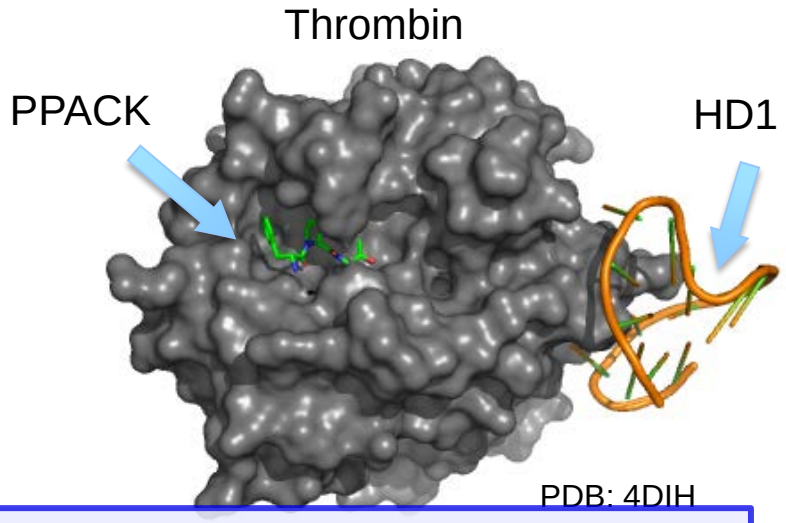




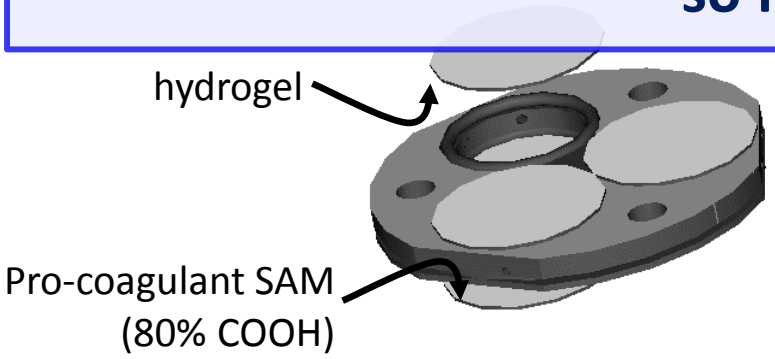


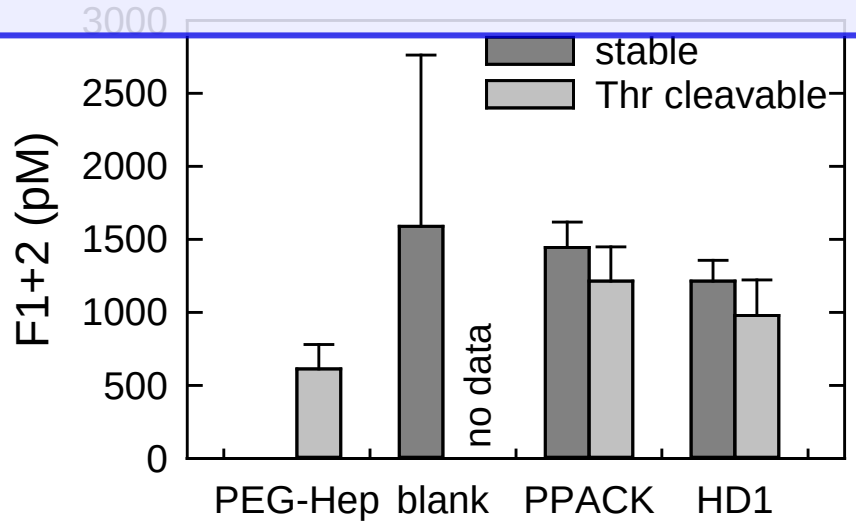
Responsive PEG hydrogels for delivery of synthetic inhibitors

- Function in whole blood



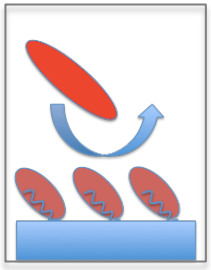
Anticoagulant activity of hydrogels with direct inhibitors –so far– is limited



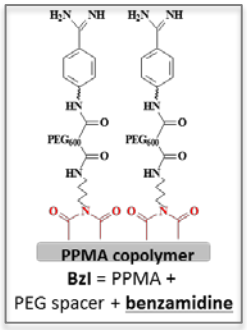


summary & perspective: surface modification for hemocompatible materials

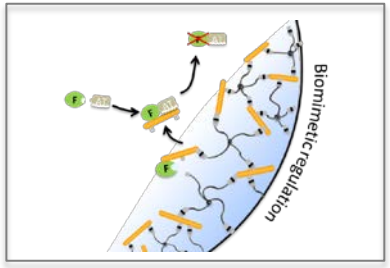
- optimization of physicochemical surface properties can be effective – but hardly sufficient



- immobilization of (e.g. coagulation) inhibitors is powerful – but difficult to dose



- **activation-controlled delivery** systems for inhibitors may create new opportunities for safety solutions with **extended durability**



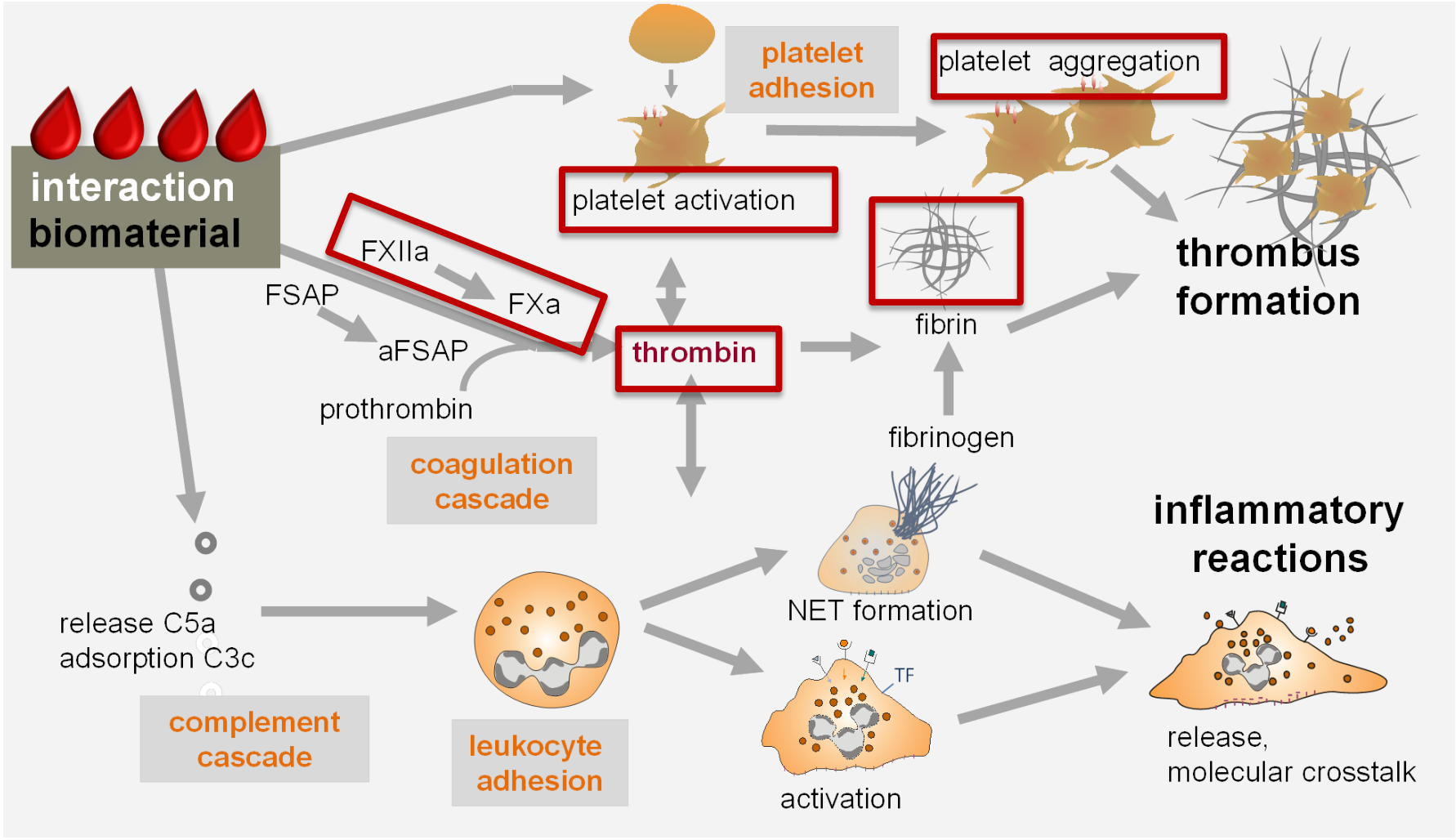
summary: comparison

passive hemocompatibility	**active hemocompatibility**
Mode of action: Mainly low interaction with blood proteins and cells	Selective interaction with activating or inhibitory systems
Smart principles of cell/protein activation may be addressed	
Usually low cost	Frequently expensive
Usually lower efficiency	High efficiency
Sterilization and shelf stability usually not critical	Sterilization and shelf stability have to be considered
Frequently long lasting biological effect	Time limited effect due to saturation or bio-degradation

summary: strategies for material coatings for passive antithrombogenicity

1. hydrophilicity
2. hydrophobicity / omniphobicity
3. antifouling polymer brushes
4. surface modifying additives

active interference of surfaces with blood



summary: targets and substances for material coatings for active antithrombogenicity

1. thrombin inhibition

coatings based on natural substances

- **thrombomodulin** (complex formation with thrombin)
- **heparin** (activation of antithrombin AT)
- **hirudin** (direct thrombin inhibition)

coatings based on synthetic inhibitors

- **small molecule synthetic inhibitors**

2. inhibition of FXIIa

corn trypsin inhibitor

3. inhibition of platelet activation and aggregation

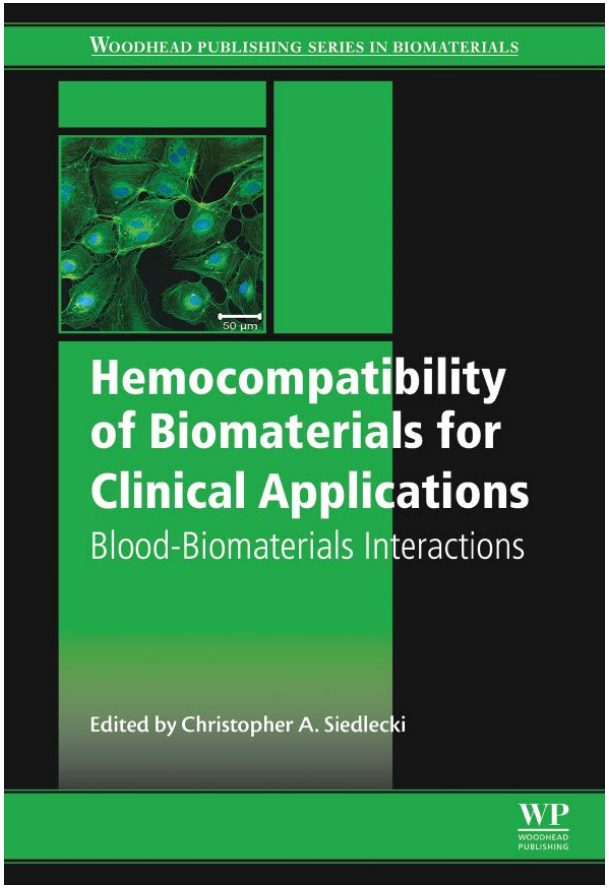
dipyridamol

nitric oxide

4. activaton of fibrinolysis

tissue-type plasminogen activator

Further reading



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C. Sperling, M.F. Maitz, C. Werner,
Test methods for hemocompatibility of biomaterials
pp. 77-104

M. Fischer, M. Maitz, C. Werner,
Coatings for biomaterials to improve hemocompatibility
pp. 163-190

in: C.A. Siedlecki (Ed.), Hemocompatibility of Biomaterials for Clinical Applications, Elsevier, Amsterdam, 2017.

http://www.ipfdd.de/2879.0.html
Passwort: surface

the hemocomp group in IPF

Claudia Sperling, Manfred Maitz,
Sandra Schulz, Tina Helmecke, Steffi Hänsel, Martina Franke,
students

Thank you for your attention!